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THE ACTION OF OXIDIZING AND REDUCING AGENTS ON FLOUR

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In previous publications from this laboratory (Sullivan, Howe, and Schmalz, 1936; Sullivan and Howe, 1937), we have shown that the presence of glutathione was responsible for the harmful action of germ on wheat flour. It would be logical to assume that low-grade flours owe their poorer baking quality, in part at least, to the presence of small amounts of germ which cannot be completely removed in milling and which contain glutathione. Indeed, by potentiometric titration, the low grade showed the presence of more reducing substances than the clear and the clear more than the patent.

It was soon apparent, however, that all flours which responded to bromate or Agene did not show the amount of reducing substance to be expected. Certain low-grade flours give a faint nitroprusside reaction (test for S−H) but the clear and especially the patent give tests that are negative or not clearly positive. Furthermore, certain mill streams such as the break flours which show an enormous response to oxidation give negative nitroprusside tests in the flour and in the dough. Titration values as shown in Table I support this. At first

TABLE I

COMPARISON OF THE BROMATE RESPONSE IN BAKE AND THE AMOUNT OF BROMATE REDUCED

Sample	Protein (13.5% moisture)	Response to $KBrO_3$ in bake	0.01 *N* $KBrO_3$ used per 20 g. sample
	%	*cc.*	*cc.*
Patent	12.90	+ 80	0.80
Clear	16.10	+370	1.05
Low grade	17.50	+350	1.50
4th midds	12.40	− 10	0.80
3rd break	16.60	+510	0.95
2nd chunks	15.90	+480	1.80
Germ (N × 6.25)	30.00	—	50.00
Bran (N × 6.25)	17.80	—	3.80

we thought that this might be attributed to the fact that glutathione, or some other substance containing a reducing group, was combined in such a way that even its qualitative identification was made impossible. There is no question but that the harmful effect of wheat germ on flour is due to its content of glutathione, but as the work progressed on flour, the problem became much more complicated. We have not been able to show that flours with poorer baking quality always have a higher glutathione content.

Jørgensen (1935), as well as Balls and Hale, believes that flour contains "powerful but latent" proteolytic enzymes and that the proteolytic enzymes are of the papain type. Papain is activated by SH compounds, hydrogen sulfide, and hydrocyanic acid, and inactivated by certain chemicals such as bromate, iodate, and ascorbic acid. Flour is weakened by the former compounds and strengthened by the use, in proper amounts, of the latter compounds. Balls and Hale (1938) have concentrated a proteolytic enzyme from an extract of wheat bran and concluded that it is an enzyme of the papain type. They have also obtained some concentration of the proteinase from flour although the latter was difficult to purify.

Jørgensen (1938) has attempted to support his theory by experiments indicating the decrease in water-soluble nitrogen of flour extracts with increasing amounts of bromate. The amounts of oxidizing agents or ascorbic acid necessary to produce any marked change in the water-soluble nitrogen are far in excess of the amounts normally used for improvement in baking quality. Jørgensen says that this is because, in such extractions, the bromate, for example, is acting in approximately one-seventh of the concentration which exists in fermenting doughs and that yeast, which activates the proteolytic enzymes, is present in the dough and gives, when present in the extraction mixture, a greater increase of soluble nitrogen with smaller amounts of bromate.

Read and Haas (1939) found that tests made on gelatin showed that the proteinase of wheat malt flour is far less responsive to inhibition by bromate than are papain and bromelin. When bromate was used in quantities applicable to commercial bakery practice, no significant repression of wheat proteinase was apparent. Ford and Maiden (1938) are of the opinion that the action of glutathione is a direct action on the gluten proteins rather than an activation of the proteolytic enzymes.

Balls and Hale (1936) state that with regard to the action of certain reducing substances on gluten, it might be that there are two processes to consider, the action of cysteine and similar compounds on the protein and on the proteolytic enzyme. Balls and Hale in a later publication

(1938) state that since the proteinase of wheat is an enzyme similar to papain, its inactivation by oxidizing agents such as bromate, persulphate, metavanadate, and Agene still seems to explain the beneficial effect of such bread improvers.

Bungenberg de Jong (1938) offers the following possible explanation. The protein system of gluten interacts with lecithin, by which action indirectly the more hydrophobic substances such as fats and sterols can be bound to the protein. Unsaturated fatty acids influence the solvation of the protein lecithin complex. This total system is sensitive to oxidation-reduction processes. Whether this is a direct action on some active group or an indirect action on the surface film has yet to be demonstrated.

Baker and Mize (1937) have presented some very interesting results on mixing doughs in vacuum and in the presence of various gases such as oxygen, nitrogen and hydrogen. Doughs mixed in vacuum or inert gases showed no deterioration from long continued mixing. Such deterioration did occur when oxygen or oxygen yielding compounds were present. The action of bromate in a dough is affected, according to Baker and Mize, by the amount of mixing a dough has received.

According to Freilich and Frey (1937) the incorporation of oxygen into bread doughs inhibits proteolytic activity of the papain type in dough. The inhibition of proteolysis was found to be directly proportional to the amount of flour treated with oxygen. When only the papain was treated with oxygen, there was practically no diminution in proteolytic effect. Baker and Mize (1939) state that in the absence of both yeast fermentation and mechanical motion bromate has little apparent action and that one of the functions of a sponge is the activation of the bromate by the motion which the fermentation produces.

Swanson and Dines (1939) in some work on the wheat-meal-time fermentation test found that since the "time" on flour is longer than on wheat meal, it would appear that the location of the protease is not in the endosperm. They also found that the addition of bran material to the flour increases instead of shortens the "time" although the bran has presumably more proteinase.

Thus it can be seen that there is considerable confusion in the literature, and that not all chemists are agreed that the effect of improvers such as bromate, iodate, Agene, ascorbic acid, and metavanadate is to be attributed to their effect in inhibiting the proteolytic enzymes, however attractive this theory may be.

Since the importance of the proteinase of flour itself has not been clearly demonstrated, and we do not know much concerning its amount or effect on the fermentation of a normal flour, a great many of the

experimental facts brought to bear on the subject have been adapted to the flour-improvement problem in reasoning by analogy as follows rather than by direct evidence. Bromate inhibits the activity of papain; HCN and SH compounds activate the enzyme. Therefore, since flour reacts in a similar way, it must contain a proteolytic enzyme of the papain type which exerts great influence. When too much of the enzyme is present, bromate inhibits the enzyme and greater gas retention and better bread are the result.

It is a foregone conclusion that in any living thing like wheat an enzyme capable of hydrolyzing the proteins under certain conditions would be present. The greatest concentration of this enzyme is probably in the aleurone layer, bran, or germ. On autolysis of the flour itself or in following the fermentation of a dough, the increases in the carboxyl or amino group are not large enough to indicate any considerable hydrolysis to amino acids. It might be argued that perhaps such degradation of the gluten to amino acids would not be necessary and that a partial disaggregation of the proteins as the first step of proteolysis would cause as much ill effect to the breadmaking quality of flours. As Bungenberg de Jong (1938) points out, one of the characteristics of such a disintegration is the quick reaction. In a flour dough an appreciable weakening of the gluten takes place only after some hours. Also, too large an amount of reductant is needed to activate the small amount of proteinase present. Furthermore, even when the proteinase is destroyed by heat an increase in soluble nitrogen is found on the addition of reducing substances.

When germ is added to flour in any appreciable amount it causes a pronounced weakening and this, we found, was due to its content of glutathione. Certain chemists have assumed that the germ acted in this manner because of its content of proteolytic enzymes or because its glutathione activated the proteolytic enzymes of the flour, and both these facts are taken as a support of the proteolytic theory. Flohil's interpretation of his experiments (1936) have supported Jørgensen's theory. Flohil believes that germ exerts its harmful effect because of its proteolytic enzymes. We have always believed that glutathione and other SH compounds acted mainly *per se* on the gluten in case of sound normal flour. The action of such compounds as glutathione, thioglycollic acid, and hydrogen sulfide is too rapid to be attributed solely to enzyme activation. Furthermore, if such compounds acted only as activators of a proteinase, the dough should become more sticky and soft as the time of fermentation increased. As Geddes (1930) has shown, the harmful effect of germ cannot be ascribed to a proteinase added with the germ since the detrimental effect progressively decreased with increasing fermentation time. We have found

that by boiling the water extract of fresh germ for 10 to 15 minutes (which should kill or at least attenuate the activity of the enzyme) the effect was practically as bad as the original unboiled extract. Of course, boiling cannot be continued to the place where glutathione is oxidized or decomposed.

It would seem to us very probable that glutathione acts for the most part directly on the gluten and that the reason that flour-germ mixtures bake better as the fermentation time increases is simply that the glutathione becomes oxidized during the course of the fermentation, consequently lessening its damaging effect.

It was formerly thought that improvers were all oxidizing agents and that damaging results to flour quality were found only with reducing agents. There are, however, certain easily reversible oxidation-reduction systems where either the oxidized or reduced form may be used to cause an improvement. Ascorbic acid-dehydroascorbic acid is one example of this and we have encountered several others.

Since the whole problem of flour improvement was so imperfectly understood in our own minds, we attempted in the beginning to eliminate as many constituents of the flour as we could. Many of the experiments are far from conclusive, but we are including some of them, believing they might be of help to other workers in this field.

The Influence of Oxidizing Agents on the Lipids

Various publications from this laboratory have been concerned with the lipids of flour and wheat germ (Sullivan and Near, 1933; Sullivan, Near and Foley, 1936; Sullivan and Bailey, 1936; Sullivan and Howe, 1938).

When flour is stored under conditions of high moisture and temperature, it may go out of condition and bake very poorly. The unsaturated fatty acids which result on hydrolysis become oxidized and are responsible for the "short" gluten, poor volume, and grain. A flour which is overoxidized by chemicals also gives an open grain and a poor volume. The overoxidization in each case, however, has a different mechanism and although some effects are similar, there are decided differences.

It has also been our experience that the ether extraction of certain flours, particularly our hard spring wheat flours, gives a tough gluten, a tough dough, and poor volume in the bread. The ether extract of the flour will bring it back to normal but the ether extract of germ, lecithin, cottonseed oil, and many other shortenings, though they give better handling characteristics to the dough, will not restore the flour to its normal condition.

Bromate and iodate both give a good response with an ether ex-

tracted flour, although the response is not so large as with the unextracted sample. This is true of the ordinary sponge or straight dough as well as a hearth bread method using a long sponge time, braking the dough, and allowing it to stand on peel for several hours. An ether extracted flour still contains some lipids, but the bromate response would lead one to believe that the lipids are not primarily responsible for the beneficial effect of these oxidizing agents. It was of interest, however, to check this further.

Doughs were made with the untreated and the bromated or iodated flour. After fermentation the gluten was washed from the doughs. The *gluten* from bromated doughs is softer than the gluten from the untreated flour, though the *dough* from the treated flour is shorter than that of the untreated sample. The glutens were analyzed and it was found that while their percentage of nitrogen did not change, their lipid contents (alcohol-ether extract)[1] were significantly different. The glutens from the bromated doughs had less lipid than the gluten from untreated doughs. The gluten from the iodated dough was shorter than those from the bromated or untreated dough. The gluten washed from the iodated dough had still less lipids than the bromated glutens. For example, the dough from the untreated flour showed a lipid content of its gluten of 6%, the gluten from the bromated flour 5%, and the gluten from the iodated dough 4%. The amounts were different depending upon the formula and the time of fermentation. Loss in lipid was greater when no sugar was used in the formula than when yeast, salt, and sugar were used. This loss of lipids in the gluten on treatment with bromate and iodate has been observed on many different crop years and with different types of flour. The longer the fermentation, the greater the loss. We do not know as yet the full explanation of these observations.

It is interesting to note that corresponding to this loss of total lipid on the gluten there is a great loss in nitrogen content of the lipid. The percentage decreases from 1.639% nitrogen on the lipid from the gluten of the untreated flour to 0.02% on the gluten lipid from the sample treated with KIO_3 as shown in Table II. Accompanying this loss of nitrogen there was also a loss of phosphorus, the weights of the yellow precipitate decreasing from 0.0918*g* to 0.0777*g*. These results were obtained with an 18 hour fermentation.

It is generally recognized that lecithin improves baking quality by making the doughs more mellow. Assuming that the loss of lipid is due to the oxidation of lecithin by the $KBrO_3$ or KIO_3, then the

[1] The sample to be analyzed (in this case the dried gluten) is weighed in an alundum cylinder and refluxed for 2 hours with 95% alcohol, then for 3 hours with ethyl ether. The combined extracts are evaporated almost to dryness (care must be taken at this stage), taken up in ether, filtered through a Bertrand filter packed with asbestos washed previously with water, alcohol, and ether and dried to constant weight *in vacuo* under 100° C.

TABLE II

EFFECT OF BROMATE ON GLUTEN

Formula	Time	Gluten recovered from dough	Lipid of gluten	Nitrogen of lipid	Phosphorus of lipid
		%	%	%	%
Flour, water	30 min.	13.2	—	—	—
Flour, water	5 hrs.	13.2	—	—	—
2% yeast, 2% salt, 2% sugar	5 hrs.	12.3	—	—	—
2% yeast, 2% salt, 2% sugar, bromate	5 hrs.	12.1	—	—	—
Flour, water	16 hrs.	12.6	—	—	—
Flour, water, bromate	16 hrs.	12.7	—	—	—
2.5% salt, 1% sugar, 0.25% yeast	18 hrs.	11.2	6.32	1.639	0.639
2.5% salt, 1% sugar, 0.25% yeast, bromate	18 hrs.	11.4	5.35	0.729	0.631
2.5% salt, 1% sugar, 0.25% yeast, iodate	18 hrs.	10.8	4.97	0.020	0.609

removal of lecithin whether by ether extraction or oxidation might tighten up the dough and cause an increase in gas retention. Therefore, an improvement in volume by the addition of $KBrO_3$ or KIO_3 would result.

It has been previously stated that the ether extract of germ will not restore ether extracted flour to its original baking status. On the other hand, the ether extract of flour when added to the ether extracted product will return to the flour the original baking characteristics. The fats from both the germ and the flour have been investigated and one of the marked differences between the two fats is that flour fat will give a positive test for loosely bound sulfur. This test is completely negative on the germ fat. This sulfur compound is found in the phosphatide fraction of the fat. The phosphatide fraction comprises 25% of the flour fat and 4.5% of the germ fat. This fraction was separated by repeated precipitations with acetone. Table III

TABLE III

COMPARISON OF FLOUR AND GERM PHOSPHATIDE FRACTION

	Flour	Germ
% ether extract precipitated by acetone	25.0	4.5
% fatty acids	46.2	—
% nitrogen	4.28	1.211
% phosphorus	1.33	2.21
% sulfur	0.7952	Negative
% sugar as glucose	2.10	1.77
Ratio N : P	7.1 : 1	1.2 : 1
Biuret test	Positive	Negative
Millon's test	Positive	Negative
Xanthoproteic test	Positive	Negative

shows a comparison of the flour and germ phosphatide fraction. Our work would indicate that the phosphatides of lecithin and cephalin are linked to a sulfur-containing peptide. This fraction has a nitrogen content of about 13% and sulfur content of approximately 2½%.[2]

Polarographic results on the lipid material from the gluten do not show the presence of any reducing substance. Likewise, the unextracted and the ether extracted flours give practically the same curves on the polarograph. These results will be reported later.

Our results have indicated that, although the lipid or lipid protein complex of the gluten is important and affected by the addition of oxidizing agents, the influences on the lipid complex by such chemicals as bromate, iodate, or Agene are not primarily responsible for the improving action of oxidizing agents on flour.

Influence of Improvers on Gassing Power and Diastatic Activity

Many experiments have been done on different flours using improvers such as bromate, iodate, and Agene to see if the gassing power of a flour was affected in any significant way. Small differences in the rate of gassing power are observed but nothing of great significance. In Table IV results are given using the pressuremeter with 10 g. of

TABLE IV

EFFECT OF POTASSIUM IODATE AND POTASSIUM BROMATE ON GASSING POWER

	Hours					
	1	2	3	4	5	6
	Millimeters of mercury					
No treatment	81	214	362	431	471	502
0.002% KIO_3	80	214	364	426	462	495
0.004% KIO_3	80	217	363	426	463	491
0.006% KIO_3	83	215	362	426	462	494
No treatment	87	220	371	437	476	506
0.002% $KBrO_3$	84	226	370	443	487	515
0.004% $KBrO_3$	84	221	372	444	486	515
0.006% $KBrO_3$	85	217	370	442	484	514

flour (at 13.5% moisture basis) and 7.0 ml. of yeast solution containing 0.3 g. yeast. A short patent (unbleached and treated with 0.1% malt) was employed. The flour was tested using three different amounts of potassium bromate and potassium iodate. No significant difference was observed except for a tendency of the iodate to give slightly lower results beginning with the fourth hour, and the bromate a slightly greater increase in gas production beginning with the fourth hour as compared with the untreated sample. Table V, using an

[2] After this paper was written an article by Balls and Hale appeared in this journal (17: 243–245, 1940) on this same subject.

TABLE V

EFFECT OF POTASSIUM BROMATE AND POTASSIUM IODATE ON GASSING POWER

Treatment	Hours					
	1	2	3	4	5	6
	Millimeters of mercury					
0.004% $KBrO_3$	95	210	339	390	418	450
0.004% KIO_3	95	209	335	374	401	421

unbleached unmalted straight treated with an equal amount of bromate and iodate, gives a further illustration of the relatively lower gas production with iodate. The same formula was used as given for Table IV. Pressuremeter results using 1% yeast and 3% salt with an untreated short patent and the same flour bromated with 0.004% and 0.016% showed no difference that could be recognized by the pressure readings as illustrated in Table VI. A high percentage of salt, of course, depresses the gassing power.

TABLE VI

EFFECT OF POTASSIUM BROMATE ON GASSING POWER USING 1% YEAST AND 3% SALT

	Hours					
	1	2	3	4	5	6
	Millimeters of mercury					
Untreated	14	48	87	128	169	208
0.004% $KBrO_3$	15	46	87	125	169	202
0.016% $KBrO_3$	15	43	83	127	163	200

On the other hand, Figure 1 shows that $KBrO_3$ under certain circumstances can affect the rate of gas production. A short patent was used with a formula of 1% yeast and 3% salt in order to slow up the fermentation so that gas production could be measured over a relatively long period. Results were obtained on the fermentograph and are corrected for loss by diffusion. It will be noted that all dosages of bromate from 0.004% to 0.012% $KBrO_3$ definitely depress the CO_2 production as compared with the untreated dough. The lowest amount of bromate has the greatest depressing effect. Figure 2 shows the same material plotted in a different way. In graphing the CO_2 per hour against the hours of fermentation the very characteristic dip is noticed in the fifth hour on this formula. Similar experiments using 3% yeast and 1% salt do not show any significant difference between the gassing power of the untreated and bromated flour. If anything, the bromated dough may give in the later hours slightly more gas.

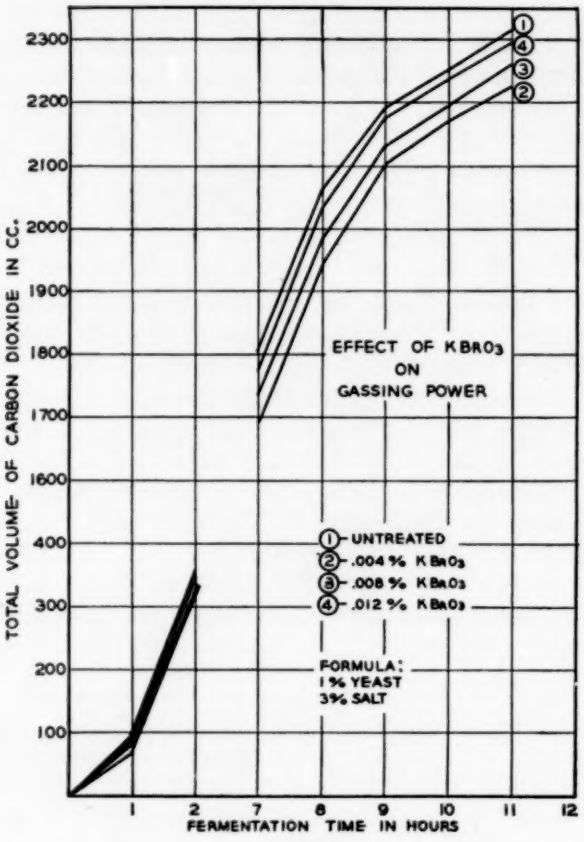


Fig. 1.

The effect of Agene on the gassing power of a spring wheat clear as measured by the pressuremeter is given in Table VII. The usual

TABLE VII

EFFECT OF AGENE ON THE GASSING POWER OF A SPRING WHEAT CLEAR

Clear flour	Hours					
	1	2	3	4	5	6
	Millimeters of mercury					
Unmalted, unbleached	87	211	267	296	319	341
Unmalted, unbleached + 0.2% malt	87	211	309	344	376	394
Unmalted, unbleached + 0.5% malt	88	217	342	380	408	436
Unmalted, unbleached + 1.0% malt	87	214	350	403	438	471
Unmalted, bleached, 10 g. agene	87	210	269	298	323	342
Unmalted, bleached + 0.2% malt	88	211	312	350	380	400
Unmalted, bleached + 0.5% malt	90	221	349	383	410	437
Unmalted, bleached + 1.0% malt	89	218	357	410	444	474

3% of yeast and no salt were employed. As will be noted in Table VII, no marked difference can be detected on either the unmalted or malted samples when bleached with Agene. Agene can modify to a slight extent the rate of gas production, however, when the latter is measured by more sensitive means.

Hence, depending on the formula, particularly the percentage of salt and yeast and the amount of treatment, bromate gave a little greater or a little less gas than untreated flour. Iodate gave slightly less gas. Agene showed no marked effect. While the rate of gas production might be changed slightly depending on the formula and

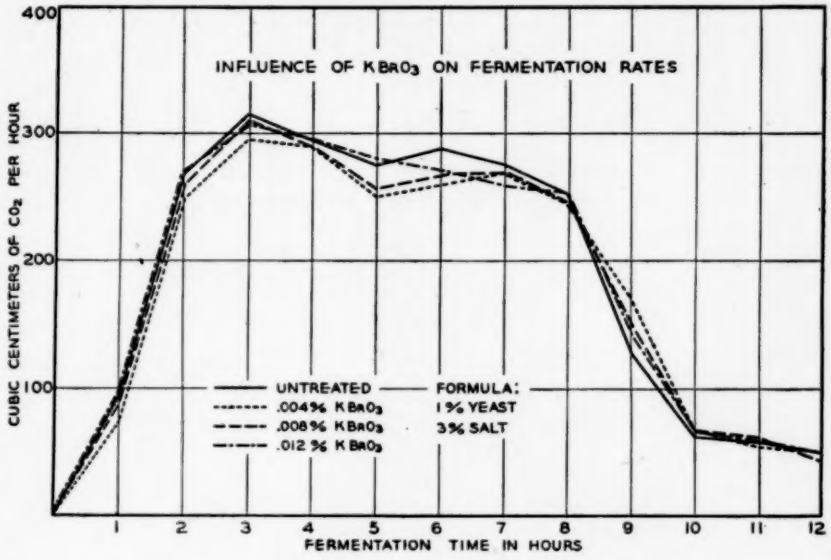


Fig. 2.

fermentation time, the beneficial effect of most of the improvers cannot be explained by any significant effect on the diastatic enzymes. Likewise, the measurement of the maltose value itself on an untreated flour and the same flour treated with Agene, bromate, iodate, metavanadate, and ascorbic acid shows no noticeable difference in the number of units.

Then it was thought that bromate, for example, might conceivably exert a catalytic effect on alpha amylase. The following experiment was tried. Fifty grams of malted wheat flour was made up to 250 cc., centrifuged, and the centrifugate heated for 15 minutes at 70° C. This treatment should kill most of the beta-amylase, according to Ohlson. A spring-wheat unbleached straight was baked alone, with 10 cc. of the alpha-amylase preparation, with 10 cc. of the alpha-

amylase preparation and 0.0047% potassium bromate, and with 0.0047% potassium bromate alone. Results are given in Table VIII. Apparently bromate under these conditions had no effect on the alpha-amylase activity.

TABLE VIII

EFFECT OF BROMATE ON ALPHA-AMYLASE

	Dough quality	Volume	Grain and texture	Color
No treatment, unbleached flour	Good, elastic	95	Fair	95
10 cc. α amylase	V.G., elastic	97	Good	96
10 cc. α amylase and 0.0047% $KBrO_3$	V.G., elastic	109½	V.G.	97c
0.0047% $KBrO_3$	V.G., elastic	110	V.G.	97

Influence of Starch, Sugars, and Fermentation

Since improvers did not seem to have any significant effect on the diastatic enzymes it was of interest to see if the sugars or the starch fraction could explain, in part at least, the action of such improvers as potassium bromate.

First of all, the amount of potassium bromate used up at the various stages of a straight dough was measured by titration with sodium thiosulfate. It was found that over half of the bromate was used immediately upon mixing the dough. This is probably due to the liberation of glutathione or other reducing substances from the yeast. A comparatively small amount of bromate was used up during the three hours of fermentation. The bread was taken from the oven and it was found that no test for bromate could be obtained although 32.9% bromate was present when the loaf went to the oven. This is illustrated below.

	Percent of total $KBrO_3$ reduced
From mixer	54.3
During fermentation	12.8
In oven 15 minutes	32.9

It appears that heat is necessary to speed up the reaction which causes the reduction of bromate.

Because of the rapid disappearance of bromate from the dough in the oven, we thought that possibly the $KBrO_3$ was oxidizing the sugars present. We made the following series of titrations using maltose and sucrose and titrating with the $Na_2S_2O_3$.

Maltose and 5 cc. 0.01*N* $KBrO_3$ at pH 5.58
Unheated—required 5.00 cc. of 0.01*N* $Na_2S_2O_3$
Heated 30′—required 2.70 cc. of 0.01*N* $Na_2S_2O_3$
Sucrose and 5 cc. 0.01*N* $KBrO_3$ at pH 5.58
Unheated—required 5.00 cc. of 0.01*N* $Na_2S_2O_3$
Heated 30′—required 3.75 cc. of 0.01*N* $Na_2S_2O_3$

We then made up two doughs with and without $KBrO_3$ and determined the amount of sucrose and maltose remaining in the bread. The following results were obtained:

	% sucrose remaining in bread	% maltose remaining in bread
Bromated dough	0.47	2.51
Regular dough	0.52	2.41

It is evident from this experiment that the bread from the bromated dough contains about the same amounts of sucrose and maltose as the bread from the untreated dough. However, it is interesting to note that bromated and iodated doughs are invariably darker in crust color than the unoxidized flour, while Agene treatment has the tendency to produce a paler crust than the untreated flour.

The following experiment was conducted to determine the extent of the starch influence. Starch and gluten were separated by washing, and the starch fraction was obtained by centrifuging the water suspension. This fraction was fermented 3 hours with yeast, salt, and $KBrO_3$. An amount of bromate equivalent to 10.8 cc. of 0.01*N* $Na_2S_2O_3$ was used.

Starch fermented 3 hours	5.08 cc. 0.01*N* $KBrO_3$ reduced
Starch fermented 3 hours, 30 minutes in oven	6.15 cc. 0.01*N* $KBrO_3$ reduced
Dough fermented 3 hours	5.95 cc. 0.01*N* $KBrO_3$ reduced
Dough fermented 3 hours, 30 minutes in oven	10.80 cc. 0.01*N* $KBrO_3$ reduced

From these experiments it can be seen that there is something present in the dough not found in the starch that reduces all the $KBrO_3$ when heat is applied. This is probably found in gluten. The starch fraction, however, like the lipids, exerts a secondary effect.

The following experiment may also be of some interest as to what occurs during fermentation. Sublimed sulfur (0.33%) was added to the ingredients of a straight dough. The dough became soft and sticky and resembled a dough which had been treated with glutathione or excess amounts of cysteine. An unmistakable odor of H_2S was emitted by the dough during fermentation. This softening of the dough and the H_2S odor are indications that a reducing medium exists in the dough of such strength that it is able to reduce sulfur to H_2S, which in turn exerts the characteristic effect of the SH group on the gluten.

The following experiment is of interest in this connection. A short patent was employed in a straight-dough process. Various amounts of sublimed sulfur were tried as illustrated in Table IX. It can be seen that here, too, 0.1% sulfur (based on the flour weight) was very damaging. The dough was short and sticky and the resultant bread had poor volume and a coarse open grain. The bread was similar

TABLE IX

EFFECT OF VARIOUS AMOUNTS OF SUBLIMED SULFUR

Flour	Treatment	Feeling of dough	Loaf volume	Grain and texture
Short patent	None	Very good, elastic	100%	Very good
Short patent	0.05% S	Good, elastic, sl. soft	96%	Good
Short patent	0.10% S	Fair, elastic, very soft	80%	Poor
Short patent	0.17% S	Poor, very soft, sticky	54%	Very poor
Short patent	0.33% S	Soft and sticky, could not be handled	—	—

to loaves made from flour to which germ or glutathione had been added. Another experiment was tried using sulfur with and without bromate as illustrated in Table X. Bromate improved the loaf considerably.

TABLE X

EFFECTS OF SULFUR WITH AND WITHOUT BROMATE

Flour	Treatment	Feeling of dough	Loaf volume	Grain and texture
Short patent	None	Very good, elastic	100%	Very good
Short patent	0.1% S	Fair, short, very soft	82%	Poor, coarse
Short patent	0.1% S 0.0045% $KBrO_3$	Good, sl. short	97%	Good plus
Short patent	0.0045% $KBrO_3$	Good, sl. tight, sl. short	93%	Fair, sl. open, tough
Short patent	0.13% cystine	Fair, sl. soft	96%	Fair, open, tough

The same amount of bromate when used in the dough without the sulfur addition showed over-oxidation of the flour. Large amounts of cystine can also be reduced, although with less effect than with the same percentage of sulfur. It is of interest in connection with sulfur that flour-water curves on the farinograph using 0.33% sulfur showed only a slight softening, indicating that time or conditions of fermentation are necessary for the reduction.

Influence of Gluten

Since neither the lipids, starch, sugars, nor diastatic enzymes appeared to be primarily responsible for the beneficial effect of certain oxidizing agents, everything seems to point to the gluten. Many factors influence the response of flour to oxidation such as climate and other growing conditions of the wheat, but it is frequently the case that the higher the protein content of the wheat the greater the bromate response of its flours and the more treatment the flour requires for best baking results. This indicates that the oxidizing effect may be primarily due to the gluten.

It was mentioned earlier that the action of certain compounds containing a sulfhydryl group was very rapid. For example 110 g. of wet gluten from 240 g. of flour was ground with 0.1 cc. of thioglycollic acid. The gluten disintegrated rapidly and became almost completely peptized. Cysteine and glutathione also cause an extreme softening and liquefaction of the gluten. In our opinion, the action of these SH compounds is not on any proteolytic enzyme because one would expect the latter to have been washed away, to some extent at least, in recovering the gluten. Furthermore, the amounts of these sulfhydryl compounds necessary to damage the bake are larger than one would expect if they acted merely as activators of the proteolytic enzyme. It is difficult to prove definitely that the activation of the proteolytic enzymes is a secondary effect until we have a better way of measuring proteolysis. The difficulty is greater because proteolytic enzymes of the papain type contain a sulfur linkage like the gluten proteins.

In order to obtain some direct proof that a reducing group could be liberated from the gluten, the gluten was washed from 30 g. of a spring wheat clear having 16.5% protein. The wet gluten was cut in very fine pieces and 2 cc. dichlorophenolindophenol (0.1 g. to 200 cc.) and 98 cc. of a buffer solution pH 5.59 added. A blank was made up using the same quantities of indicator and buffer. On heating on the steam bath, the gluten flask became colorless in about an hour while the blank remained the same original shade of blue. Different experiments of this nature showed the presence of a reducing substance on heating or on standing a considerable time, but the experiments were unsatisfactory and could not be made quantitative because only the surface of the gluten was affected.

The following experiment may be of some interest. A straight grade flour (unbleached and unmalted) having 0.46% ash and 14.10% protein (calculated at 13.5% moisture) was doughed up using ingredients given below:

Treatment	Yeast	Salt	Sugar
	%	%	%
1. None	2	2	None
2. 0.005% $KBrO_3$	2	2	None
3. None	2	2	5
4. 0.005% $KBrO_3$	2	2	5
5. None	None	None	None

Dough No. 5 (flour and water) was washed out after standing 30 minutes. The other doughs were handled as follows: 1st punch, 1 hour 45 minutes; 2nd punch, 30 minutes; to bench, 20 minutes; to oven, 1 hour 25 minutes later. After this total of four hours of fermentation, the glutens were washed out and analyzed. The glutens

washed from a fermented dough start out being short, then milky, and always end up shorter than the original flour-water gluten. The bromated doughs are always tougher or shorter than the unbromated, but the *gluten* washed from these bromated doughs is more extensible than the gluten from the untreated dough. The percentage of gluten recovered and the analyses of the glutens are given in the table below.

Experiment No.	Dry gluten from doughs %	Moisture %	Protein (N × 5.7) %	Lipid %	Ash %
1	12.5	1.43	90.10	6.75	.34
2	12.4	1.53	90.50	5.37	.29
3	12.2	1.60	91.00	6.92	.25
4	12.3	1.80	90.75	6.00	.25
5	13.3	1.51	89.10	7.01	.36

As has been mentioned before, some of the gluten cannot be recovered after fermentation although that which can be recovered is, as a rule, in good shape unless the fermentation time is very long. The longer the fermentation time, the more difficult it is to recover the gluten because of its extreme shortness.

Because the amount of starch held by these glutens was so small, it was not measured. Better than 7% of the total gluten was lost during fermentation. As pointed out under lipids, a greater loss of lipids was encountered in all the fermented doughs than in the case of the flour-water dough which stood only 30 minutes (Experiment No. 5). Also, the bromated dough lost more lipid than the untreated dough and the dough containing no sugar lost more lipids than the 5% sugar dough. There is no significant difference in the protein content of the glutens.

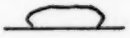
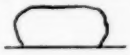
The experiments thus show that both the chemical analysis and physical properties of a gluten which has undergone fermentation are changed.

Influence of Bakery Machinery

The amelioration of overoxidation by apparently purely physical means is most peculiar. Bromate and iodate, when used in more than optimum amounts in an ordinary formula, have a tendency toward shortening or lessening the elasticity of a dough. Freilich and Frey (1939) have shown that an overtreated dough showing signs of shortness and age can be brought back by remixing. We have long observed that the shortness and age shown in a treated flour can also be overcome in part by putting the dough through a dough brake several times.

A short patent (0.39% ash, 12.80% protein) was baked with no bromate and 0.0045% bromate using the following formula: yeast, 2%;

salt, 2%; sugar, 4%; malt, 1%; dry skimmilk, 3%; lard, 3%. Absorption was 62%. First punch was given after 1 hour and 45 minutes, second punch after 30 minutes. The doughs were left 25 minutes and then panned or broke. After molding or braking, the doughs were placed on boards and allowed to remain 19 hours. The appearance after this time is roughly illustrated below.

	Treatment	Appearance
1. No $KBrO_3$	Hand molded	
2. 0.0045% $KBrO_3$	Hand molded	
3. No $KBrO_3$	Machine molded	
4. 0.0045% $KBrO_3$	Machine molded	
5. No $KBrO_3$	Dough brake 10 times	
6. 0.0045% $KBrO_3$	Dough brake 10 times	

The largest loaf by far when part of the same dough was baked was No. 6.

The amount of bromate which produces the best loaf on molding and handling, according to the normal punching and fermentation time, is less than the amount that will produce the biggest volume with a brake dough.

The return to normal on remixing or braking of an overtreated dough is difficult to explain other than on the basis that some sort of a redox system is present in the dough or by the purely physical changes in the dough structure resulting from mechanical handling.

Discussion

Many of the well known flour improvers have outstanding differences in their mechanism of reaction in flour doughs even though the end result of all of them is an improvement in some such factor or combination of factors as absorption, fermentation tolerance, handling characteristics, or the volume, grain, or texture of the finished bread.

As is well known, any given flour which responds to an improver demands a certain critical amount of the improver for any given formula and procedure. Overtreatment can produce worse results than no treatment at all. For best baking results a flour must have a certain definite oxidation potential whether it has obtained it from the wheat or by artificial treatment.

No two flour improvers exert their effect in exactly the same way. For instance, iodate reacts very differently from bromate. The action

of iodate is very rapid. An iodated dough feels tighter immediately after mixing. On the other hand, a bromated dough feels no different from the mixer than the untreated sample. Bromate does not exert its effect in any physical manner until sometime after fermentation has started. An explanation of this fact lies in the different pH requirements for the oxidizing actions of bromate and iodate. It is known that in order to exert its oxidizing effect iodate requires less acidity than bromate. A few experiments as given below using buffer solutions of various pH were done to illustrate this difference in behavior.

To 100 ml. of the buffer solution was added 20 ml. of 0.01*N* potassium bromate or 20 ml. of 0.01*N* potassium iodate together with 10 ml. of 30% potassium iodide. After a time interval (20 to 30 minutes) starch solution was added and the liberated iodine was titrated with 0.01*N* sodium thiosulfate.

pH	Oxidized by $KBrO_3$	Oxidized by KIO_3
4.0	No	—
5.0	No	Yes
5.5	No	Yes
6.0	No	Yes
6.5	No	No

A longer time interval would probably have given the bromate a chance to exert its oxidizing effect. Also, a different reducing substance such as cysteine of glutathione might have been oxidized by bromate at the pH levels of dough. The above tabulation, in any case, shows that potassium iodate acts as an oxidizing agent more readily and at higher pH levels than potassium bromate. This can be illustrated also by the following experiment. A sample of flour was divided and one portion treated with 0.002% $KBrO_3$ and another with 0.002% KIO_3. A water extract of each of these flours was made and KI, H_2SO_4, and starch indicator solution added in the proper amounts. In the case of the water extract from the bromated flour, one can obtain a titration with thiosulfate but none can be obtained with the iodated flour. This indicates that the KIO_3 is entirely reduced while the $KBrO_3$ is not completely reduced.

On the other hand, iodate is similar to bromate in that it will make a dough which has been run through a dough brake; hold gas over a long period on the peel and give a good volume, whereas Agene and ascorbic acid will not. Iodate must always be used in smaller amounts than bromate for the same effect. Vanadate, likewise, gives results similar to bromate and iodate but in still smaller amounts than iodate. Vanadate and iodate when used in a sponge do not give the characteristic appearance of breaking as will the untreated or bromated

doughs. Copper salts, either cuprous or cupric, will likewise give good results on a long time lean dough but must be used in an amount approximately double that of bromate.

Agene and bromate act in a different manner also in that Agene treatment can often be felt immediately from the mixer and on the washed gluten from a treated flour, whereas bromate cannot except after long standing.

It is known that bromate plus minute amounts of vanadate has a beneficial effect on doughs and enables less bromate to be used. We assume that this is due to the fact that the reaction rate between SH (or a similar reducing group of the protein molecule) and vanadium must be faster than the rate between bromate and the reducing group. It is conceivable that the SH group reduces the vanadium in sodium metavanadate to V^{++}. The V^{++} is oxidized to V^{+++++} and the SH reduces the V^{+++++} to V^{++} again when the cycle may continue until the equilibrium is thrown too far to one side. It is important to remember that the $SH \rightleftarrows S-S$ is not an easily reversible system under all conditions. The H ion as a rule is rather easily detached from an SH group.

The explanation of the action of certain chemicals in improving the baking quality of flour hinges on the proof that SH or some similar reducing group is present in the dough. A complete proof of this is needed before the proteolytic theory or our own could be accepted. Assuming the fact that a reducing group is present in the protein and is fairly readily dissociable, the following mechanisms could be offered in explanation of the beneficial effect of certain improvers. Certain other possible theories are also discussed.

Some flours have more of a reducing substance than others. During fermentation the dehydrase of the yeast liberates hydrogen from this substance. The active H^+ is then combined with oxygen which has been activated by an oxidase or a metal (Cu or V) or by some oxidizing agent capable of a steady supply of active oxygen at the pH of dough. Cu or V are able to cause a definite electron transfer which the organic substances present cannot do. The H^+ released from the reducing substance then could form H_2O_2 which would be acted on by catalase to form water and oxygen as follows:

$$2H + 2O \xrightarrow[\text{oxidase}]{} H_2O_2 \xrightarrow[\text{catalase}]{} H_2O + 1/2O_2.$$

Oxidase is inhibited by bisulfite, HCN, and certain other reducing substances. A flour which responds to $KBrO_3$, KIO_3, Agene, etc., has a higher content of reducing substances or substances capable of giving up H^+ under influence of dehydrases. A flour not responding

has only a small amount of such H^+ donating substrates. The oxidation reduction process goes on until the H^+ donator is used up in cases where oxygen is supplied by bromate, iodate, or mixing. In the case of Agene the probability is that NCl_3 combines with the oxidases to stop the action at one certain stage, thus preventing the further continuation of the oxidation reduction system.

Another theory may be considered more likely. There are present in gluten certain S−H groups bound to other protein linkages or S−S groups capable of reduction to SH. During fermentation some of these sulfhydryl groups dissociate and can be oxidized by oxygen from the air or from oxidizing agents such as bromate or iodate. The rate of oxidation is dependent on the degree of dissociation of the SH groups from the gluten and the oxidation potential of the fermentation. Certain metals such as Cu can form mercaptides and this system as well as the $2SH \rightleftarrows S-S + 2H$ system is in a certain measure reversible. Many other systems such as ascorbic-dehydroascorbic acid act as oxygen carriers. Agene, no doubt, oxidizes the SH more or less irreversibly.

The alteration of the gas-retaining capacity of a dough by purely physical means such as mixing, punching, molding, and braking as well as by certain chemicals is caused by a change in the protein structure. Our work has given strong indications that some change in the labile sulfur linkages of the gluten can explain a number of the problems of flour chemistry. Such reactions are responsible for the change in the physical characteristics of certain doughs and the improvement given by oxidizing agents or oxidation-reduction systems.

If we assume that the gluten strands are coiled fibrils, it is conceivable that a main or side chain containing R−S−S−R may be acted on by oxygen and water (or agents capable of supplying oxygen) to form $R(SO)_2R$, RSO_3H or RSH etc., whereby the sulfur linkage would be altered or broken. If a scission of the molecule should take place, it would result in shorter strands, less extensibility and greater "shortness." Similarly certain reducing agents might attack the sulfur linkage not causing a hydrolytic cleavage but only the uncoiling of the long fibrils producing greater softness and extensibility but less elasticity. It is possible to imagine that in the first instance the broken fibrils may be knitted together or aligned to some extent by purely physical means such as braking a dough. The effect of reducing agents is usually a greater softness as well as shortness of the dough. The softness or too great extensibility may be overcome to some extent by punching or molding since such physical procedures would coil back the untwisted strands if too much damage had not been done to the sulfur linkage of the protein molecule.

Needless to say, such a theory is difficult to prove because the sulfur compounds are not present in a free state and therefore their isolation is difficult. Even on pure compounds such as cystine, the mechanism of its oxidation and reduction is very imperfectly understood. However, the effect of oxidizing and reducing agents on flour seems to be explained best with the facts available at present on the basis of their direct effect on the sulfur linkage of the flour proteins.

Conclusion

Several experiments bearing on the action of oxidizing and reducing agents on flour and in dough have been described. A few theories have been presented as possible explanations. All the work indicates that changes in the sulfur linkages of the gluten proteins are responsible for many of the effects described.

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THE RELATION BETWEEN THE NORMAL FARINOGRAM AND THE BAKING STRENGTH OF WESTERN CANADIAN WHEAT [1]

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(Read at the Annual Meeting, May 1939)

When the Brabender farinograph was introduced on the American continent it was suggested (Brabender, 1934) that the relative strength of different flours could be predicted from measurements of the curves produced by the instrument. More recently the original claims made for the instrument have been modified and Munz and Brabender (1940) now write: "The farinograph is adapted to the measurement of dough plasticity as a function of continuous mixing. This instrument has also proved successful in estimating the capacity of a strong flour to yield doughs of acceptable properties when blended with weak flour. From the characteristics of farinograms, other dough properties, such as the rate of hydration, sensitivity to mixing, and buckiness, can be estimated. The farinograms are of limited value in certain other particulars, however. Thus, they fail to reveal the direction and magn tude of the effect of chemical treatments, or the recovery or tendency of the dough to regain certain of its original properties after excessive mixing." In connection with this quotation it is worth noting that most of the current literature on the farinograph deals with studies which are not primarily concerned with its utility for predicting baking

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strength. The majority of the papers describe applications of the instrument to such problems as the determination of absorption, calibration of dough mixers, the estimation of proteolytic activity and studies on the effect of starch, dried gluten, milk solids and germ constituents on the colloidal properties of wheat-flour doughs.

In 1935, when the work described in the present paper was started, there were some who believed that measurements of the physical properties of doughs might replace the baking test as a rapid method of assessing baking quality,though published data bearing on this hypothesis were negligible in amount and many cereal technologists did not support the idea. Since that time statistical studies of the relations between the physical properties of doughs and baking characteristics have been made by Markley and Bailey (1939). These workers found that protein content was highly correlated with both flour absorption and dough-development time and that, in a series of 23 spring-wheat flours, the correlation between protein content and loaf volume was much higher than that between the decrease in consistency upon extended mixing in the farinograph and the volume of the test loaves. They also reported that the increase in mobility of doughs upon prolonged mixing was not significantly correlated with the decrease in loaf volume resulting from similar treatment.

Incidental to the routine testing of Western Canadian hard red spring flours, it has been the practice of the Board of Grain Commissioners' Laboratory to make normal farinograms whenever time permitted and a considerable volume of data has been accumulated. In view of the limited information in the literature on the relation between loaf volume and characteristics of the normal farinogram, statistical studies of a portion of these data are now reported in this paper.

In a preliminary investigation, the results obtained with the 300-g. and 50-g. Brabender mixers were compared using five blended flours of graded strength and three dough-consistency levels, namely 500, 600, and 700 Brabender units. This investigation provided information not only on the relative performances of the two sizes of mixers but also on the effects of widely different baking strengths and different dough consistencies on the characteristics of the normal farinograms.

In the major investigation, protein determinations, baking tests, and farinograms were made on a total of 333 samples of Western Canadian hard red spring wheat flours, arranged in four series, each of which was studied separately. This investigation permitted a statistical study of the correlations between flour protein, absorption and loaf volume, on the one hand, and certain measurable characteristics of Brabender curves, on the other.

Methods

Baking.—In the main study, the experimental material comprised long-patent flours experimentally milled from Western Canadian hard red spring wheat, and the samples were baked by the malt-phosphate-bromate formula using the procedure outlined in the A. A. C. C. basic baking test (Geddes, 1934). This formula differs from that of the basic formula in that 0.3% diastatic malt (250° Lintner), 0.1% $NH_4H_2PO_4$, and 0.001% $KBrO_3$ are added as extra ingredients. The doughs are mixed for three minutes at medium speed in the Hobart mixer equipped with two dough hooks and baked in low-sided tins. Extensive experience in determining the relative strength of Canadian hard red spring wheat involving tests on untreated and only slightly aged experimentally milled flours has shown that it is necessary not only to provide for adequate gas production but also to employ an oxidizing agent to assist in proper development of the gluten. Numerous trials with various baking formulas and procedures (*e.g.* Aitken and Geddes, 1934) led to the adoption of the above formula for routine testing purposes since it gives wide differentiations between flours; the loaf volumes for commercially grown varieties and the various wheat grades are also in line with their accepted evaluation by Canadian and overseas millers.

Brabender farinograms.—Aside from the preliminary study involving comparisons of the 50-g. and 300-g. Brabender mixers, the small mixer was used throughout. Fifty grams of flour (13.5% moisture basis) was mixed at 30°C. for 15 minutes at 600 farinograph units, a preliminary curve being run to determine the required amount of water.

For statistical purposes, it is necessary to secure quantitative values representing the curve characteristics, the most significant of which seemed to be (1) the "dough-development time" or mixing time required to produce a dough of maximum consistency, (2) the decrease in consistency upon overmixing, and (3) the mean width of the curve after maximum dough development. At the time these studies were initiated, no methods for evaluating these curve characteristics had been proposed and the following measurements, as illustrated in Figure 1, were arbitrarily selected.

1. Dough-development angle is the angle between the line drawn from the midpoint of the curve at maximum dough development to where the curve meets the 400-unit line and this latter line.

2. Weakening area is the area in square centimeters as measured by a planimeter, enclosed by a line drawn from the midpoint of the curve at maximum dough development to the end of the curve (15 minutes total mixing time) parallel to the 600-unit line, and from this point

down to the midpoint of the end of the curve and returning to the dough-development point.

3. Mean band width is the total area of the curve (in square centimeters), omitting irregularities, from the point of maximum dough development to the end of the curve (15 minutes total mixing time), divided by the length of the median line of this portion of the curve.

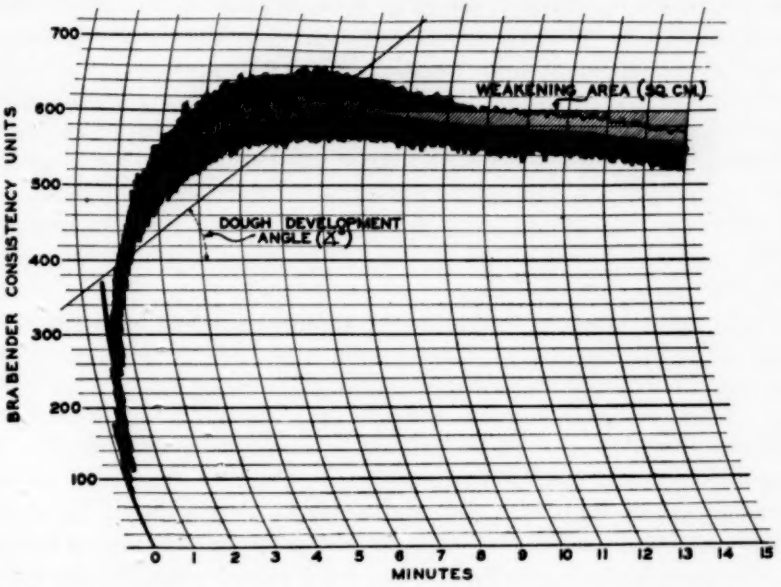

Fig. 1. Normal farinogram, showing the methods employed in securing quantitative measures of dough-development time (dough-development angle), mobility increase upon overmixing (weakening area), and mean band width.

The dough-development angle was originally selected as a measure of dough-development time because it was felt that it could be determined with greater precision than the direct estimation of this value from the farinograms. This choice was unfortunate since such indices of dough-development time for curves made at different mobility levels are not directly comparable and the values for any particular level are subject to error where the midpoint of the band at maximum dough development does not fall precisely on the mobility level selected; moreover the angle is inversely related to dough-development time, and this is somewhat confusing. In the later series of measurements, the dough-development time as read from the farinograph was also recorded and employed in the statistical studies.

I. Comparison of Large and Small Mixers—Effect of Absorption and Dough-Consistency Level on Farinograph Curve Characteristics

At the time the farinograph was acquired, the small or 50-g. mixer had just been introduced and the manufacturers recommended that

the large or 300-g. mixer should be used whenever possible; moreover, it was suggested that the "dough consistency" at 500 units, recommended for use in Europe, might not be entirely satisfactory for the stronger flours with which the authors had to deal. Accordingly, before employing the farinograph as a routine testing instrument, a preliminary study was undertaken to determine the relation between the two mixers in regard to curve type and replicability of results and also as an indication of the most desirable consistency level to employ to secure the optimum differentiation between samples.

For this purpose, a suitably graded series of flours was obtained by using a high-protein Canadian spring wheat baker's patent and a low-protein cake flour, together with three blends of these. Farinograms on each of these five flours were made in quadruplicate in both the large and small mixers at dough-consistency levels of 500, 600, and 700 units. Measurements of the curves were made in duplicate and submitted to statistical analysis.

Both mixers readily differentiated between the flours in regard to dough-development time and weakening area. While the two mixers gave different absolute values for the curve characteristics with the same flours, the general trends with changing flour strength were quite similar. The changes in dough-development time and weakening area for the extremes in strength represented in this series were, on the average, slightly higher for the small mixer but the replicability of the tests in the large mixer tended to be somewhat better. It was noteworthy that the flour absorptions required to yield doughs giving the same consistency values in the two mixers differed materially. The mean difference for all consistency levels varied from 0.6% for the weakest flour to 2.8% for the strongest flour. The importance of these differences in relation to the use of the farinograph as a standard method for determining absorption will not be discussed at this time but it should be mentioned that the instruments used in the present study were purchased before it became the standard practice of the manufacturers to calibrate all mixers against a standard machine at 500 units.

In spite of the fact that the larger mixer appeared to be somewhat superior with respect to replicability, it was decided to adopt the small mixer for all routine studies. The replicability and differentiations obtained with the latter were quite adequate for most purposes and it has the added advantage of requiring only one-sixth the quantity of flour per test, which is of particular importance in this laboratory where the size of sample available for study is frequently limited.

The most suitable mobility level to employ in routine studies was not conclusively indicated by the preliminary study. The character

of the curve for any particular flour is materially influenced by the consistency level employed. Very stiff doughs give a short and fairly precise dough-development time, a wide band and a rapid break-down or decrease in consistency upon further mixing. As the consistency is lowered, the curve band becomes narrower and the decrease in consistency much less rapid; at low consistencies strong flours actually give a rising curve for the entire 15-minute mixing period, thus giving a long and indeterminate dough-development time and a negative weakening area. At the 500-unit level, the increase in mobility with extended mixing is relatively slight for strong flours and it is difficult to estimate the point of maximum dough development with accuracy since the curve usually has a zero or very slight negative slope for an appreciable interval of time. The 700-unit consistency gave the greatest range in the mean weakening area for the various flours, but the replicability was in general not as satisfactory as for the curves made at 600-units consistency. On the whole, it appeared that the intermediate level of 600 units would prove most satisfactory for the flours ordinarily tested in the Laboratory, and this level was accordingly used in further investigations reported in this paper.

II. Relation between Curve Characteristics and Loaf Volume

While there are wide differences in the types of normal farinograms given by strong and weak flours when mixed to the same maximum consistency, it is of interest to determine whether, with flour of the same general type, curve characteristics such as development time, width of curve, and decrease in consistency upon overmixing are related to baking strength (as indicated by loaf volume determined by the malt-phosphate-bromate baking formula), or reflect other properties not related to baking strength. It is also of interest to ascertain the relative degrees of association between these individual curve characteristics and loaf volume.

It is well known that oxidizing agents, such as potassium bromate, have pronounced effects on the baking properties of flours, yet such flours yield normal farinograms essentially similar to the corresponding untreated samples. Since commercial flours may vary widely in the extent of treatment with maturing agents, no general relation between the baking results and curve characteristics of such flours can be expected. Such relations might, however, be found with samples of untreated flours of similar history and such samples provide experimental material most favorable to the farinograph for determining its possible value as an index of baking strength. Flours experimentally milled from different grades of Canadian wheat are, therefore, very suitable for investigating the usefulness of the farinograph in this

connection. They are classed on the world's markets as strong and medium-strong flours and the various grades from which they are milled exhibit differences in average strength which are universally recognized by the domestic and foreign trade. Such general commercial evaluations are useful in a study of this kind, where the integrity of the experimental baking test as a standard of reference may be questioned by some workers.

The relations between farinograph and baking data were studied for four series of untreated long-patent experimentally milled flours of approximately equal extraction representing: (1) 215 samples of wheat cargoes of the 1935–36 crop year; (2) 48 samples of plant breeders' varieties of the 1935 crop; (3) 30 samples of Winnipeg inspection office averages for different grades representing three crops, 1936, 1937, and 1938; and (4) a frosted wheat series representing 40 samples of No. 3 and No. 4 Northern wheat collected for an investigation of the effect of frost damage on milling and baking characteristics. (See Malloch, Geddes, Larmour, and McCalla, 1937.)

Cargo series.—The minimal, maximal, and mean values for flour protein, loaf volume, absorption at 600 Brabender units, and farinogram measurements are recorded in Table I together with the standard deviations of the individual values; the results of variance analyses, expressing the significance of the differences in the respective mean values for the three grades, are also summarized by the F values and their 5% points.

The differences in mean protein content and loaf volume for the three grades are highly significant while the absorption values are closely similar. The means for dough-development angle follow essentially the same trend as those for loaf volume and protein content but the mean weakening areas for the various grades are not significantly different and those for band width differ but slightly and do not show a consistent trend with grade.

The simple correlation coefficients given in Table II show that loaf volume, dough-development angle, and weakening area are significantly correlated with protein content. The correlation between protein and loaf volume (+.903) is significantly higher than the correlations between protein content and dough-development angle (−.735) and protein content and weakening area (−.652). In interpreting the correlations involving dough-development angle, it should be recalled that this measure is inversely related to dough-development time. The correlation between loaf volume and dough-development angle (−.699) and that between loaf volume and weakening area (−.619) are not significantly different but are significantly lower than the correlation between loaf volume and protein content (+.903).

TABLE I

STATISTICAL CONSTANTS FOR FLOUR PROTEIN, ABSORPTION, AND FARINOGRAPH MEASUREMENTS ON CARGO SAMPLES

	No. 1 Northern 82 samples	No. 2 Northern 60 samples	No. 3 Northern 73 samples	All grades 215 samples
MINIMA				
Flour protein, %	12.7	11.0	10.5	10.5
Loaf volume, *cc.*	852	742	558	558
Absorption (at 600 B.U.), %	55.0	54.6	55.2	54.6
Dough-development angle degree	26.5	22.0	23.5	22.0
Weakening area, *sq. cm.*	1.1	0.2	0.2	0.2
Mean band width, *cm.*	0.93	0.50	0.82	0.50
MAXIMA				
Flour protein, %	14.5	15.1	14.0	15.1
Loaf volume, *cc.*	1030	992	940	1030
Absorption (at 600 B.U.), %	60.6	61.6	61.2	61.6
Dough-development angle degree	47.0	50.0	69.5	69.5
Weakening area, *sq. cm.*	7.3	6.0	12.0	12.0
Mean band width, *cm.*	2.30	2.80	1.43	2.80
MEANS				
Flour protein, %	13.30	13.04	12.08	12.81
Loaf volume, *cc.*	932.4	877.0	744.0	853.0
Absorption (at 600 B.U.), %	57.97	58.64	58.08	58.19
Dough-development angle degree	33.72	35.30	44.16	37.71
Weakening area, *sq. cm.*	3.25	2.78	4.79	3.64
Mean band width, *cm.*	1.13	1.20	1.19	1.17
DIFFERENCES BETWEEN GRADES				
	F.			*5% pt.*
Flour protein, %	118.93			3.18
Loaf volume, *cc.*	56.62			3.18
Absorption (at 600 B.U.), %	0.68			3.18
Dough-development angle degree	12.74			3.18
Weakening area, *sq. cm.*	0.64			3.18
Mean band width, *cm.*	4.04			3.18
STANDARD DEVIATIONS (OF INDIVIDUAL VALUES)				
Flour protein, %	0.34	0.74	0.66	0.78
Loaf volume, *cc.*	40.27	66.11	88.47	105.20
Absorption (at 600 B.U.), %	1.28	1.88	1.49	1.58
Dough-development angle degree	5.63	7.50	10.61	9.34
Weakening area, *sq. cm.*	1.41	1.58	3.22	2.39
Mean band width, *cm.*	0.10	0.14	0.14	0.13
COEFFICIENTS OF VARIABILITY				
Flour protein, %	2.6	5.7	5.5	6.1
Loaf volume, %	4.3	7.5	11.9	12.3
Absorption (at 600 B.U.), %	2.2	3.2	2.6	2.7
Dough-development angle, %	16.7	21.2	24.0	24.8
Weakening area, %	43.4	56.8	67.2	65.7
Mean band width, %	8.6	11.9	11.3	11.1

TABLE II

SIMPLE CORRELATION COEFFICIENTS AND LINEAR REGRESSION EQUATIONS INVOLVING FLOUR PROTEIN, LOAF VOLUME, AND FARINOGRAM MEASUREMENTS—CARGO SERIES

	Absorption	Loaf volume	Dough development angle	Weakening area	Mean band width
SIMPLE CORRELATION COEFFICIENTS [1]					
Flour protein	−.173	+.903	−.735	−.652	+.222
Loaf volume	—	—	−.699	−.619	+.167
Dough-development angle	—	—	—	+.830	−.354
Weakening area	—	—	—	—	−.549

LINEAR REGRESSION EQUATIONS AND STANDARD ERROR OF PREDICTION (*cc.*)	
Loaf volume = −717.9 + 122.63 flour protein	45.2
Loaf volume = 1149.9 − 7.87 dough-development angle	75.3
Loaf volume = 952.4 − 27.31 weakening area	82.7
Loaf volume = 693.0 + 136.76 mean band width	103.7

[1] Value of r at 5% point = +.138 (approx.).

The correlation between loaf volume and band width (+.167) is not significant and that between protein content and band width (+.222) only slightly exceeds the 5% level of significance. Mean band width is then neither materially influenced by protein content nor is it related to loaf volume. The high correlation between dough-development angle and weakening area (+.830) would be anticipated from the general form of farinograms for flours of varying strength, since increasing dough-development time is generally associated with decreasing weakening area.

The significantly higher correlation between loaf volume and protein than between loaf volume and dough-development angle or weakening area implies that protein content is the most reliable index of flour strength in this series of samples. This can be illustrated in another way by calculating the standard errors for the prediction of loaf volume from protein content and each of the farinogram measurements. These turn out to be 45 cc. for protein content as compared with 75 and 83 cc. for dough-development angle and weakening area respectively.

In view of the close similarity between the correlation for protein content and dough-development angle and the correlation for loaf volume and dough-development angle, and the similarity between the corresponding correlations involving weakening area, it appeared that the correlations between these farinograph measures and loaf volume might be largely a reflection of protein content. That this is actually true was shown by calculating the partial correlation coefficients, inde-

pendent of protein content, for loaf volume and each of these farinogram measurements. These are recorded in Table III.

TABLE III

COMPARISON OF SIMPLE, PARTIAL, AND MULTIPLE CORRELATION COEFFICIENTS—CARGO SERIES

SIMPLE VS. PARTIAL CORRELATION COEFFICIENTS

	Simple	Partial independent of protein	Third farinograph measurement
Loaf volume and dough-development angle	−.699	−.120	—
Loaf volume and weakening area	−.619	−.092	—
Loaf volume and mean band width	+.167	−.084	—
Dough-development angle and weakening area	+.826	+.681	+.813
Dough-development angle and mean band width	−.354	−.241	+.219
Weakening area and mean band width	−.549	−.547	−.489
Value of r at 5% point	+.138 (approx.)	+.138 (approx.)	+.138 (approx.)

SIMPLE VS. MULTIPLE CORRELATIONS

	Simple	Multiple
Loaf volume and protein	+.903	—
Loaf volume and protein including dough-development angle	—	+.904
Loaf volume and protein including weakening area	—	+.904
Loaf volume and dough-development angle	−.699	—
Loaf volume and dough-development angle, including weakening area	—	+.703
Loaf volume and dough-development angle, including weakening area and mean band width	—	+.705
Value of correlation at 5% point	+.138 (approx.)	+.212 (approx.)

Since the partial correlation coefficient for loaf volume and dough-development angle is not significant (−.120), whereas the simple correlation coefficient (−.699) is significant, it is apparent that the latter reflects only the correlations between each variable and protein content, and does not represent a real relation between loaf volume and dough-development angle. Similar associations exist between protein content, loaf volume, and weakening area. The partial correlation coefficient for the last two variables, independent of protein content, is not significant (−.092), thus showing that no real relation exists between loaf volume and weakening area.

Since the partial correlation coefficients are not significant, it is apparent that the prediction of loaf volume from protein content cannot be improved upon by including farinograph measurements as additional independent variables. This is shown by the comparison of simple and multiple correlations recorded in Table III. The multi-

ple correlation (+.904) between loaf volume, on the one hand, and protein content and dough-development angle on the other hand, is not significantly greater than the simple correlation coefficient for loaf volume and protein content alone (+.903). Similar results are obtained if weakening area rather than dough-development angle is introduced as the second independent variable. It is of interest to note also that the prediction of loaf volume from either dough-development angle or weakening area cannot be improved by including the alternative farinogram measure.

The associations between loaf volume and protein content, loaf volume and dough-development angle, and loaf volume and weakening area, which have just been discussed, are based on the data for all three grades considered as a single series. However, when these relations are studied by means of the scatter diagrams given in Figures 2, 3, and 4, it appears that the No. 3 Northern samples are differentiated from those representing Nos. 1 and 2 Northern as regards the general relations between loaf volume and these three variables. The loaf volume of the No. 3 Northern samples is not as high as would be indicated by the regression equation for loaf volume on protein content, computed for the entire series, and in the instance of dough-development angle and weakening area, the differentiation of No. 3 Northern is even more clearly indicated. This suggests that a protein "quality" factor is entering into the relationships. It would, in fact, be expected that the No. 3 Northern samples would not only be of lower average "gluten quality" but would also exhibit wider variations in gluten characteristics than the samples representing Nos. 1 and 2 Northern, since, in the crop year 1935–36, Garnet wheat was admitted into this grade. It is well known that, among other differences, this variety has gluten characteristics which distinguish it from the varieties of the Marquis type which are eligible for the highest grades. The Garnet content of the No. 3 Northern samples in this series, as determined by growing tests, varied from 0.0% to 64.9% with an average of 30.8%.

It would be expected, therefore, that wider variations in protein "quality" would occur in No. 3 Northern than in Nos. 1 and 2 Northern, and it seemed possible that the farinograph might show up to better advantage, relative to protein content, as an index of loaf volume, in this particular grade. Accordingly, the data for Nos. 1 and 2 Northern combined, and for No. 3 Northern, were analyzed separately; the results of these analyses are recorded in Table IV. The simple correlation between loaf volume and protein content (+.869) for the No. 3 Northern samples is significantly higher than that for loaf volume and dough-development angle (−.738); the correlation between loaf

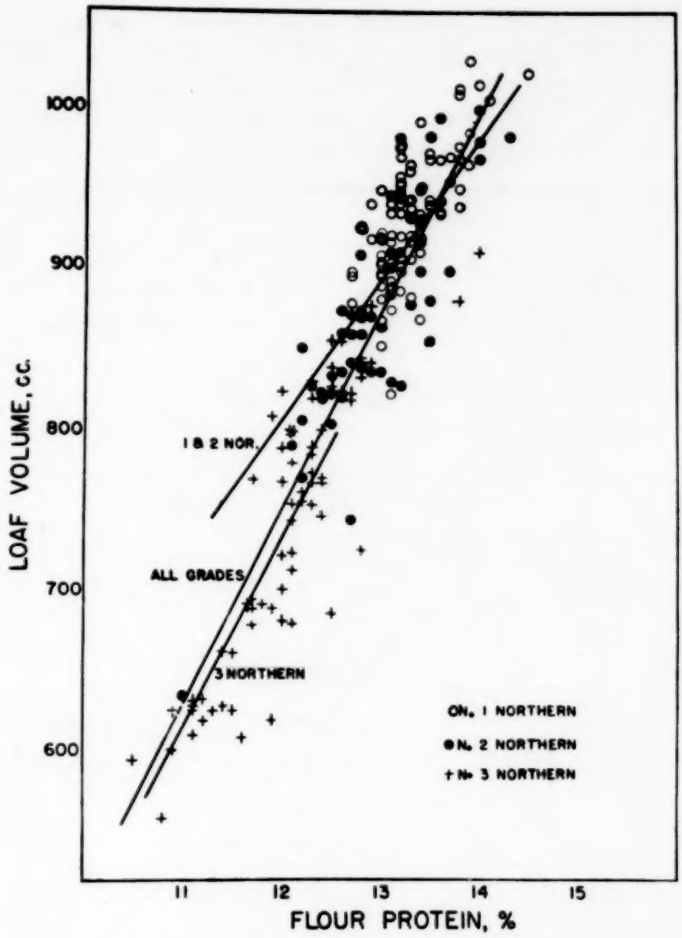

Fig. 2. Scatter diagram for loaf volume and protein content—Cargo series.

volume and weakening area (−.769) is not significantly lower than that between loaf volume and protein content although it approaches the 5% level of significance. It can at least be said that the farinograph possesses no advantage over protein content as an index of loaf volume, even in this grade where it would be expected to show up most favourably.

The complicating influence of the relations between the different variables and protein content was again removed by calculating partial correlation coefficients, independent of protein content. Low but significant partial correlation coefficients were obtained for loaf volume and each of the farinograph measures. Accordingly, it might be anticipated that there would be a slight gain in the precision with

which loaf volume could be estimated from protein content by including farinogram measurements as additional independent variables. Comparisons of the multiple correlation coefficients for the combined effects of protein content and dough-development angle on loaf volume (+.879), and the corresponding multiple correlation including

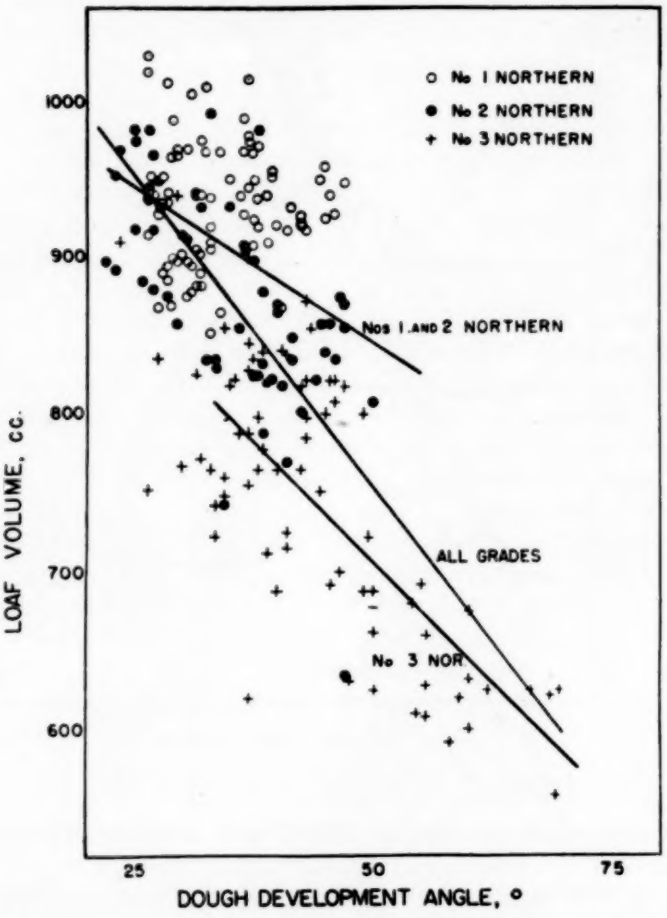

Fig. 3. Scatter diagram for loaf volume and dough-development angle—Cargo series.

weakening area (+.893), with the simple correlation between loaf volume and protein content (+.869), suggest some improvement in prediction. However, an analysis of variance showed that the multiple correlations were not significantly higher than the simple correlations and it can only be concluded that the accuracy of estimating loaf volume from protein content is not increased by including either farinograph measure.

Other series.—Similar studies were conducted on the three additional series of hard red spring wheat samples previously mentioned. In these studies measurements were made of both dough-development time, the measurement recommended by Brabender, and of dough-development angle, the corresponding measurement devised in this laboratory. On the other hand, the measurement of band width was

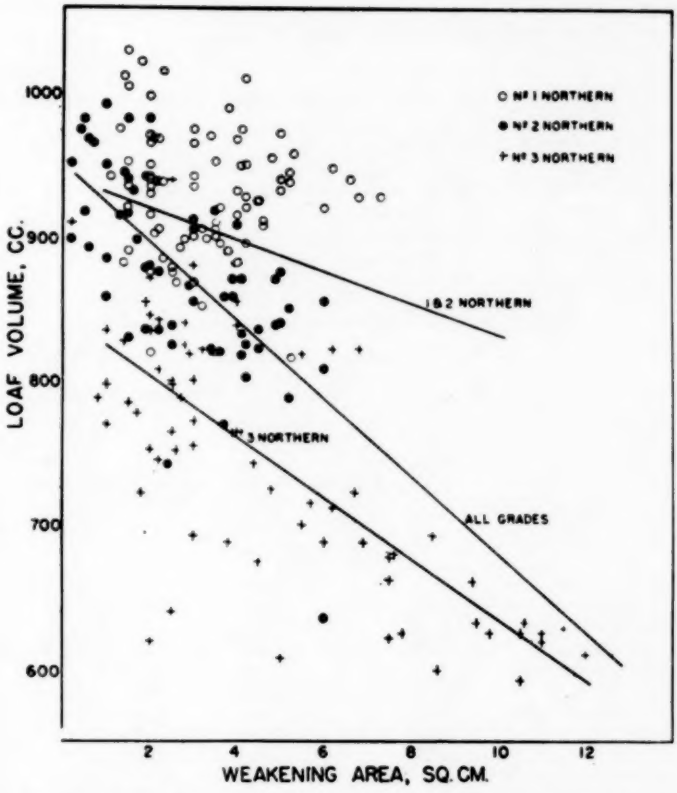

Fig. 4. Scatter diagram for loaf volume and weakening area—Cargo series.

not made, since it was time-consuming and had been proved relatively unimportant in the Cargo series.

The Plant Breeders' variety series consisted of 48 composites, representing certain named varieties and wheat hybrids grown in 1935 in rod rows at various points in the Prairie Provinces. In testing these varieties, all baking tests were conducted in quadruplicate and hence the loaf-volume data utilized in the statistical studies represent the means of four determinations rather than two as in the instance of the other series reported in this paper.

TABLE IV

STATISTICAL CONSTANTS FOR GRADES NO. 1 AND NO. 2 COMBINED AND GRADE NO. 3 NORTHERN, CARGO SERIES

	Mean		Standard deviation	
	No. 1 & No. 2 Northern	No. 3 Northern	No. 1 & No. 2 Northern	No. 3 Northern
Flour protein, %	13.2	12.1	0.52	0.66
Loaf volume, *cc.*	909.0	744.0	59.27	88.47
Dough-development angle, *degrees*	34.4	44.2	6.51	10.61
Weakening area, *sq. cm.*	3.0	4.8	1.52	3.22

LINEAR REGRESSION EQUATIONS AND STANDARD ERRORS OF PREDICTION

		cc.
	No. 1 to No. 2 Northern	
Loaf volume = −232.4 + 86.56	flour protein	38.7
Loaf volume = 1047.1 − 4.015	dough-development angle	55.3
Loaf volume = 942.5 − 10.96	weakening area	56.6
	No. 3 Northern	
Loaf volume = −663.1 + 116.48	flour protein	43.8
Loaf volume = 1045.6 − 6.15	dough-development angle	59.7
Loaf volume = 845.2 − 21.13	weakening area	56.6

SIMPLE VS. PARTIAL CORRELATION COEFFICIENTS

	No. 1 and No. 2 Northern			No. 3 Northern		
	Simple	Partial		Simple	Partial	
		Independent of:			Independent of:	
		Protein	Other farinograph measure		Protein	Other farinograph measure
Loaf volume × flour protein	+.758	—	—	+.869	—	—
Loaf volume × dough-development angle	−.358	−.316	−.232	−.738	−.265	−.321
Loaf volume × weakening area	−.281	+.156	−.024	−.769	−.414	−.442
Protein × dough-development angle	−.206	—	—	−.749	—	—
Protein × weakening area	−.488	—	—	−.722	—	—
Dough-development angle × weakening area	+.822	—	—	+.800	—	—
Approx. value of r at 5% point	—	+.159	—	—	+.232	—

SIMPLE VS. MULTIPLE CORRELATION COEFFICIENTS

	No. 1 and No. 2 Northern		No. 3 Northern	
	Simple	Multiple	Simple	Multiple
Loaf volume and protein	+.758	—	+.869	—
Loaf volume and protein, including dough-development angle	—	+.786	—	+.879
Loaf volume and protein, including weakening area	—	+.765	—	+.893
Loaf volume and dough-development angle	−.358	—	−.738	—
Loaf volume and dough-development angle, including weakening area	—	+.359	—	+.796
Approx. value of correlation at 5% point	+.159	+.244	+.232	+.351

The "Winnipeg averages" comprise composites made up of small portions of the wheat of similar grade taken from cars inspected at Winnipeg during the first few weeks of the crop movement. These samples, which represent many hundreds of cars, are collected and tested each year in connection with the annual report of the laboratory on the quality characteristics of the various grades of the new crop. In view of the nature of these samples, it seems worth while to present the individual data for protein content, loaf volume, and farinograph measurements for the crops studied (1936, 1937, and 1938); these are recorded in Table V.

TABLE V

WINNIPEG AVERAGES OF DIFFERENT GRADES: FLOUR PROTEIN CONTENT, LOAF VOLUME AND FARINOGRAPH DATA

Lab. No.	Grade	Absorption at 600 B.U.	Dough development		Weakening area	Flour protein	Loaf volume
			Time	Angle			
		%	*Min.*	*Deg.*	*Sq. cm.*	%	*cc.*
			1936 CROP				
2278	1 Hard	57.8	7.6	33.5	4.0	14.3	945
2279	1 Nor.	58.4	7.0	35.0	2.8	14.4	985
2280	2 Nor.	58.8	6.6	38.5	2.8	14.2	993
2281	3 Nor.	57.8	7.0	33.0	2.9	13.6	940
2282	4 Nor.	57.8	7.6	31.5	2.9	13.9	920
2283	1 C. W. Gar.	52.0	6.0	36.0	2.8	11.5	638
2284	2 C. W. Gar.	52.0	6.0	35.0	0	11.5	642
			1937 CROP				
4927	1 Hard	58.3	6.5	39.0	3.9	14.1	874
4928	1 Nor.	58.3	5.8	41.5	5.0	13.4	844
4929	2 Nor.	58.0	6.4	36.0	3.6	13.0	795
4930	3 Nor.	57.0	5.8	41.5	3.4	12.9	774
4931	4 Nor.	56.1	5.0	44.5	3.7	12.4	774
4932	No. 5	56.2	5.0	43.0	5.4	12.2	753
4933	4 Special	55.7	4.8	44.0	5.0	11.7	704
4934	5 Special	55.3	4.0	51.0	9.2	11.8	713
4935	1 C. W. Gar.	56.6	5.0	46.0	5.7	12.5	672
4936	2 C. W. Gar.	58.1	6.0	39.5	5.9	13.0	690
			1938 CROP				
6094	1 Hard	59.0	5.2	47.0	6.0	13.1	851
6095	1 Nor.	59.1	5.0	44.5	5.2	13.6	866
6096	2 Nor.	58.1	6.5	35.5	2.8	13.2	882
6097	3 Nor.	57.9	5.6	41.0	3.2	13.1	843
6098	4 Nor.	56.7	6.5	34.5	3.7	13.4	867
6099	No. 5	56.7	4.2	49.5	7.6	12.2	725
6100	No. 6	55.9	4.4	48.5	7.2	11.9	742
6102	4 Special	54.1	5.5	39.0	4.5	13.0	804
6103	5 Special	53.9	6.0	36.5	3.5	13.0	813
6104	6 Special	53.5	4.0	49.5	5.3	12.7	818
6105	1 C. W. Gar.	58.1	4.2	50.5	5.9	11.8	598
6106	2 C. W. Gar.	59.3	4.5	46.0	7.0	11.7	612
6107	3 C. W. Gar.	59.1	3.8	51.5	9.2	12.0	626

The frosted wheat series consists of a portion of the 1935 crop samples collected in connection with a study of the quality and grading of frosted wheat (Malloch, Geddes, Larmour, and McCalla, 1937).

The statistical constants for the three series of samples are summarized in Table VI and require little comment. The high correlations between dough-development time and dough-development angle in all three series and the close similarity in the numerical values for the correlations between these respective measures and other variables indicate that the dough-development angle, utilized in the Cargo series, was an adequate and reliable measure of the dough-development time.

Flour absorption is not significantly correlated with dough-development time, weakening area, or loaf volume, but there is a low but significant correlation between this variable and protein content in the Plant Breeders' and Winnipeg Average series. Markley, Bailey, and Harrington (1939) found high and significant correlations between absorption and these variables in a diverse group of 89 flours. These investigations indicated that the relation between absorption and dough-development time was probably nonlinear. Previously, Bailey (1930) emphasized the curvilinear relation between plasticity of doughs and the water used in their formation and mentioned that the logarithm of the plasticity plotted against the percentage of water approached a straight line. In the present study, the authors were primarily concerned with the associations between loaf volume, protein content, and farinograph measures, and the nature of the relations involving absorption has not been studied in detail.

The correlations between dough-development time and weakening area are of similar magnitude in all three series and the degree of association between these two variables and loaf volume does not differ statistically; these relations are in agreement with those noted for the Cargo series.

In all three series protein content is positively correlated with loaf volume, the coefficient for the Plant Breeders' variety series being the lowest. This may be due in part to the relatively high protein content and narrow range between samples and is probably also a reflection of differences in gluten characteristics in such hybrid material.

In the frosted wheat series the spread in protein content is fairly wide and a fairly high correlation coefficient is therefore obtained. It should be noted that in this series the gluten characteristics of the samples are probably very similar since it is well known that "bran frost," the type of damage prevailing in these samples, has little effect on baking strength, as indicated by loaf volume. In the Winnipeg average series, while the lower grades and Garnet grades differ materially in gluten quality from the higher grades, the very wide range in

TABLE VI

Statistical Constants for Plant Breeders' Varieties, Winnipeg Averages, and Frosted Wheat Series

Series	Absorption	Dough development: Time	Dough development: Angle	Weakening area	Flour protein	Loaf volume
	%	*Min.*	*Deg.*	*Sq. cm.*	%	*Cc.*
MEANS						
Plant breeders' varieties	58.37	6.13	40.61	3.84	14.72	921.00
Winnipeg averages	56.85	5.58	41.40	4.67	12.84	790.10
Frosted wheat	60.79	6.00	42.59	4.84	12.26	677.40
STANDARD DEVIATIONS						
Plant breeders' varieties	1.63	1.21	6.63	2.10	0.86	75.93
Winnipeg averages	2.04	1.08	6.05	6.43	0.88	112.50
Frosted wheat	1.94	3.16	17.22	3.80	1.56	125.23
COEFFICIENTS OF VARIABILITY						
Plant breeders' varieties	2.8	19.7	16.3	54.7	5.8	8.2
Winnipeg averages	3.6	19.4	14.6	137.7	6.9	14.2
Frosted wheat	3.2	52.7	40.4	78.5	12.7	18.5
SIMPLE CORRELATION COEFFICIENTS						
Plant breeders' varieties [1]						
Absorption	—	+.213	—	−.227	+.336	+.173
Dough-development time	—	—	−.949	−.781	+.286	+.084
Dough-development angle	—	—	—	+.822	−.131	−.052
Weakening area	—	—	—	—	+.193	−.009
Flour protein	—	—	—	—	—	+.538
Winnipeg averages [2]						
Absorption	—	+.145	—	+.076	+.460	+.319
Dough-development time	—	—	−.952	−.754	+.735	+.690
Dough-development angle	—	—	—	+.826	−.566	−.558
Weakening area	—	—	—	—	−.393	−.445
Flour protein	—	—	—	—	—	+.915
Frosted wheat series [3]						
Absorption	—	−.089	—	+.119	+.147	−.088
Dough-development time	—	—	−.922	−.671	+.827	+.852
Dough-development angle	—	—	—	+.664	−.835	−.853
Weakening area	—	—	—	—	−.723	−.757
Flour protein	—	—	—	—	—	+.871

LINEAR REGRESSION EQUATIONS

		Error of prediction
		Cc.
Plant breeders' varieties	Loaf volume = 222.5 + 47.45 protein	64.0
Winnipeg averages	Loaf volume = −715.3 + 117.24 protein	45.4
Frosted wheat series	Loaf volume = −179.8 + 69.92 protein	61.5
Winnipeg averages	Loaf volume = 388.9 + 71.88 dough-development time	81.4
Frosted wheat series	Loaf volume = 474.82 + 33.76 dough-development time	65.6
Winnipeg averages	Loaf volume = 826.4 − 7.78 weakening area	100.7
Frosted wheat series	Loaf volume = 798.2 − 24.97 weakening area	81.8

SIMPLE VS. FIRST-ORDER PARTIAL CORRELATION COEFFICIENTS

	Plant breeders' varieties		Winnipeg averages		Frosted wheat series	
	Simple	Partial holding protein constant	Simple	Partial holding protein constant	Simple	Partial holding protein constant
Loaf volume and protein	+.538	—	+.915	—	+.871	—
Loaf volume and dough-development time	+.084	−.087	+.690	+.062	+.852	+.478
Loaf volume and weakening area	−.009	−.137	−.445	−.229	−.757	−.374
Approx. value of correlation at 5% point	+.288	+.288	+.361	+.367	+.304	+.325

[1] At 5% point r = +.288 (approx.).
[2] At 5% point r = +.361.
[3] At 5% point r = +.304 (approx.).

TABLE VI—*Continued*

SIMPLE VS. MULTIPLE CORRELATION COEFFICIENTS

	Plant breeders' varieties		Winnipeg averages		Frosted wheat series	
	Simple	Multiple	Simple	Multiple	Simple	Multiple
Loaf volume and protein	+.538	—	+.915	—	+.871	—
Loaf volume and protein plus dough-development time	—	+.543	—	+.915	—	+.902
Loaf volume and protein plus weakening area	—	+.550	—	+.919	—	+.890
Loaf volume and dough-development time	+.084	—	+.690	—	+.852	—
Loaf volume and weakening area	−.009	—	−.445	—	−.757	—
Loaf volume and dough-development time plus weakening area	—	+.122	—	+.693	—	+.887
Approx. value of correlation at 5% point	+.288	+.430	+.361	+.538	+.304	+.470

protein content masks the interfering effects of quality differences, and a high correlation coefficient is obtained.

One would anticipate that if the farinograph curve characteristics reflect baking strength, they would prove most closely associated with loaf volume in Plant Breeders' material where protein quality may be expected to vary most widely. It might even be anticipated that the associations would prove to be of a higher degree than that between loaf volume and protein content. In the Plant Breeders' series, however, the correlations between loaf volume and dough-development time (+.084) and between loaf volume and weakening area (−.009) are not significant. Thus expectations are not realized. In the frosted wheat series, on the other hand, the correlations for loaf volume and dough-development time (+.852), and for loaf volume and weakening area (−.757), compare quite favourably with that between loaf volume and protein content (+.871). Corresponding correlation coefficients for the "Winnipeg averages" hold an intermediate position. The correlation for loaf volume and protein content (+.915) is significantly higher than those for loaf volume and dough-development time (+.690) or weakening area (−.445), but the last two coefficients are significant and fairly high. The result is that loaf volume may be more accurately predicted from protein than from either farinograph measure in all series. It should be noted, however, that in the frosted wheat series the correlation between loaf volume and dough-development time is not significantly lower than the correlation between loaf volume and protein content.

The partial correlation coefficients show, as in the Cargo series, that the associations between farinogram measurements and loaf volume reflect mainly the relations between each variable and protein content. When the effects of differences in protein content are removed by calculating partial correlation coefficients, these are found to be insignificant except in the frosted wheat series in which low but significant values

are obtained. However, the multiple correlations again reveal that no significant increase in precision in estimating loaf volume from protein content can be obtained by inclusion of dough-development time or weakening area. Moreover, the estimation of loaf volume from dough-development time or weakening area is not improved by the combination of both farinograph measures.

Discussion

These statistical studies of the relations between protein content, normal farinogram characteristics, and loaf volume on 333 samples of experimentally milled hard red spring wheat flours, indicate quite clearly that with such material protein content is on the whole a better index of loaf volume, as determined by the routine testing procedure employed in this laboratory, than any of the farinograph measures. The mean band width is only very slightly influenced by variations in protein content and is not significantly correlated with loaf volume. In this connection it is of interest to note that Markley (1937) found that starch-water systems gave quite broad lines in the farinograph and Markley and Bailey (1938) conclude that the band width is essentially a function of the mobility of the dough and is not a measure of its elasticity. While the dough-development time and weakening area are, respectively, positively and negatively correlated with loaf volume, the variations in these measures are largely a reflection of differences in the protein content of the flours and they give, on the whole, lower correlations with loaf volume than that between protein content and loaf volume.

It has been well established that protein content is the major factor affecting the baking capacity of Canadian hard red spring wheat. That variations in gluten quality may diminish this relation has been shown by Malloch, Geddes, Larmour, and McCalla (1937) in extensive investigations of the milling and baking quality of frosted wheat. These investigators found that the correlations between protein content and loaf volume within grades were higher than for all grades combined, indicating that the correlation is affected by the proportion of damage in the sample and that the relation can be improved if this is taken into account.

If the characteristics of the normal farinogram reflect both protein content and protein "quality," it might be expected that the prediction of loaf volume could be made more accurately from dough-development time or weakening area, or from both, than from protein content alone. This is not so, and while there are indications in the present study that the characteristics of the farinograph curve are influenced to some extent by the character of the gluten, the effects on the curve are not

sufficient to render the farinograph of any practical advantage, when combined with protein determinations, in estimating baking strength as reflected by loaf volume.

The question arises as to which of the two farinogram characteristics, dough-development time or weakening area, is more closely related to loaf volume. In all four series of samples the correlations between these measures and loaf volume were not significantly different but it is of interest to note that the numerical values for the correlations involving dough-development time (or angle) were usually the higher. Moreover, in the Cargo series, the mean weakening areas for the three grades were not significantly different and it would appear that, on the whole, dough-development time is the preferable value to use as it is much the more readily determined. Markley and Bailey (1938a) have pointed out that the rate of increase in the mobility of flour doughs upon overmixing is a function of many factors including mechanical degradation of the gluten structure, proteolytic activity, the thixotropic nature of starch in the presence of water, the action of alpha-amylase, the presence of embryo constituents, and the influence of baking ingredients. In these circumstances, it is apparent that weakening area is affected by many factors and it cannot be expected to provide a simple and direct measure of flour strength.

Although baking strength, as reflected by loaf-volume-producing ability, is the most important factor contributing to the baking value of Canadian wheats, it must be borne in mind that, in the commercial evaluation of world wheats, other factors are also taken into account. Among these, dough characteristics are of considerable importance. Moreover, in wheats of the same general type, dough characteristics may vary independently of baking strength. This tendency is frequently exhibited by samples representing different varieties of the same type of wheat grown at the same station. On the other hand, in bulk shipments of commercial grades, which represent composites of similar varieties grown under different environmental conditions, there is little indication of independent variations in baking strength and dough properties.

It is clear that the normal farinogram is less useful than protein content for estimating the baking strength of Western Canadian hard red spring wheat. The question arises, however, as to whether the farinograph provides useful information concerning dough characteristics. The present investigation does not bear directly on this problem, but the authors, after several years' work with the machine and a careful consideration of the literature on it, are forced to conclude that it has not yet been established that the farinograph is of any use in assessing such differences in dough characteristics as may occur in

flours made from the same general type of wheats, as for instance Western Canadian hard red spring wheats. Nevertheless, this possibility cannot yet be wholly discounted.

On the other hand, it appears that without possessing the merits originally claimed for it, the farinograph still retains a position as a useful laboratory tool. It has proved, for example, a useful guide in estimating flour absorption. The instrument also appears to be of value in estimating the optimum mixing time, both in the experimental laboratory and the commercial bakery. Markley, Bailey, and Harrington (1939) have suggested that in the experimental baking test it would be advisable to employ a variable rather than a fixed mixing time, and this seems worthy of careful investigation.

In addition to determining optimum mixing time, the farinograph might also prove useful in determining mixing tolerance, including the tendency of doughs to become sticky. With a little experience, the point where stickiness develops is easily detected from the farinogram, and this property of doughs materially influences the shape of the curve. Indeed, it is quite possible that the relatively poor correlation between weakening area and loaf volume may be due in part to this factor. While the correlations between loaf volume and dough-development time were not significantly higher than those for loaf volume and weakening area, it is of interest to note that the numerical values for the former were higher in all series except for the No. 3 Northern Cargo samples.

Summary

The 300- and 50-gram farinograph mixers gave similar normal curve characteristics with flours of varying strength, but the replicability of the tests in the large mixer was somewhat better.

Normal farinograph curve characteristics are greatly influenced by the dough-consistency level. Increasing the consistency at which the doughs were mixed shortened the dough-development time (*i.e.*, the mixing time required to produce doughs of maximum consistency) and increased the width of the curve and the rate at which the consistency decreased upon overmixing. The 500-unit level was unsatisfactory for strong flours and the 600-unit level was arbitrarily selected for routine studies.

Statistical studies of the relation between loaf volume, flour protein content, and farinograph curve characteristics (determined in the small mixer) on 333 samples of Western Canadian hard red spring wheat revealed higher correlations between loaf volume and protein content than between loaf volume and quantitative measures of the dough-development time, consistency decrease, and mean band width

during "overmixing." Mean band width was not significantly correlated with loaf volume and gave only a low correlation with protein content for the 215 samples where this measure was employed.

Dough-development time and consistency decrease were highly and negatively correlated. Dough-development time and consistency decrease were, respectively, positively and negatively correlated with protein content and with loaf volume. Partial correlations, independent of protein content, between these farinograph measures and loaf volume were either not significant or of a low order of magnitude, thus revealing that the correlations between loaf volume and the farinogram measurements are largely a reflection of the correlations between protein content and loaf volume, on the one hand, and protein content and farinogram measurements on the other. Low but significant partial correlations, independent of protein content, between loaf volume and the farinograph measures were obtained in the series in which variations in gluten "quality" probably existed. However, such differences as were measured by the farinograph were not reflected by loaf volume, so that the precision with which loaf volume could be estimated from flour protein content could not be improved significantly by the inclusion of the farinograph measures as additional independent variables.

Correlations for dough-development time and loaf volume, and for consistency decrease and loaf volume, were not significantly different, and the prediction of loaf volume could not be improved by including both farinogram measures.

Since the normal farinogram is of less value than protein content for estimating the loaf-volume capacity of Western Canadian hard red spring wheat flours, its utility would appear to be largely that of providing accessory information on such properties as absorption, optimum mixing time and mixing tolerance.

Acknowledgments

The authors are indebted to B. Frisell, whose services were loaned by the Brabender Corporation, Duisburg, Germany, for the preliminary experiments on the effect of absorption and dough-consistency level on the curve characteristics. They also gratefully acknowledge the assistance of Nancy Milton in connection with the statistical reduction of the data.

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DOUGH IMPROVEMENT STUDIES. II. THE EFFECT OF ADDING OXIDIZED GLUTATHIONE TO WHEAT-FLOUR DOUGHS

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Through the oxidation by bromate of the reduced glutathione (to be referred to as GSH) present in a dough, a certain amount of oxidized glutathione (to be referred to as GSSG) will exist in that dough. The present study was undertaken to see whether this GSSG itself has any effect on dough properties. The reduced glutathione used was obtained from the firm of Hoffmann-La Roche in Basle, Switzerland. The oxidation was made by titration with a $n/100$ iodine solution.

The extensimeter tests were carried out with the old type of Chopin extensimeter, but with the new kneader, with which a dough membrane can be obtained without touching the dough (Chopin, 1937). Kent-Jones (1939) has described the two kinds of apparatus, giving at the same time an explanation of the meaning of the results obtained. Duplicate extensimeter tests on nonfermenting doughs from 400 wheats carried out by Avard and Ugrimoff (1938) gave an average difference of 6% for the factor W, a measure of the mechanical work done. According to Scott Blair and Potel (1937), W depends partly on the tendency of the doughs to allow gas to escape through it before rupture.

They also state that P is related to viscosity and should be a measure primarily of water-absorbing capacity, whereas G is dependent on both "spring" and "shortness."

Experimental

The curves obtained with the extensimeter for one series are given in Figure 1. There are certain difficulties in handling fermenting

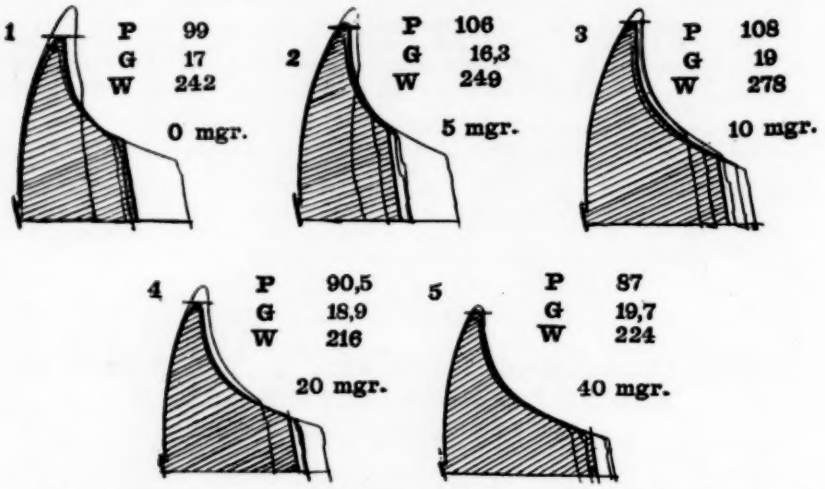

Fig. 1. Extensimeter tests. Doughs made with 250 g. commercial short patent flour, 2% salt, 3% yeast and 50% water. Mixed 8 minutes. Fermentation 25 minutes at 25°C. Amount of GSSG given in mg. per 250 g. flour.

doughs in this test which explain the divergence in the results that arise more from the difficulty of measuring G than P. One effect was that with increasing increments of GSSG the dispersion in the height (P) and length (G) of the five curves decreased. This would be expected if fermentation had been suppressed by the addition, but this was not the case as baking and gassing tests, to be referred to later, showed.

The results of two independent series of extensimeter tests using the same sample of short patent flour are shown in Figure 2, which demonstrates clearly that the tenacity (P) of the fermenting dough and the amount of mechanical work needed (W) both go through a distinct maximum on the addition of 4 mg. GSSG per 100 g. flour. With larger amounts the dough still retains more spring and is less "short" than the original untreated dough. Bromate, on the contrary, renders dough "short."

Figure 3 brings out the effect, as measured by the farinograph, of the addition of GSSG to a fermenting dough. The arrow indicates

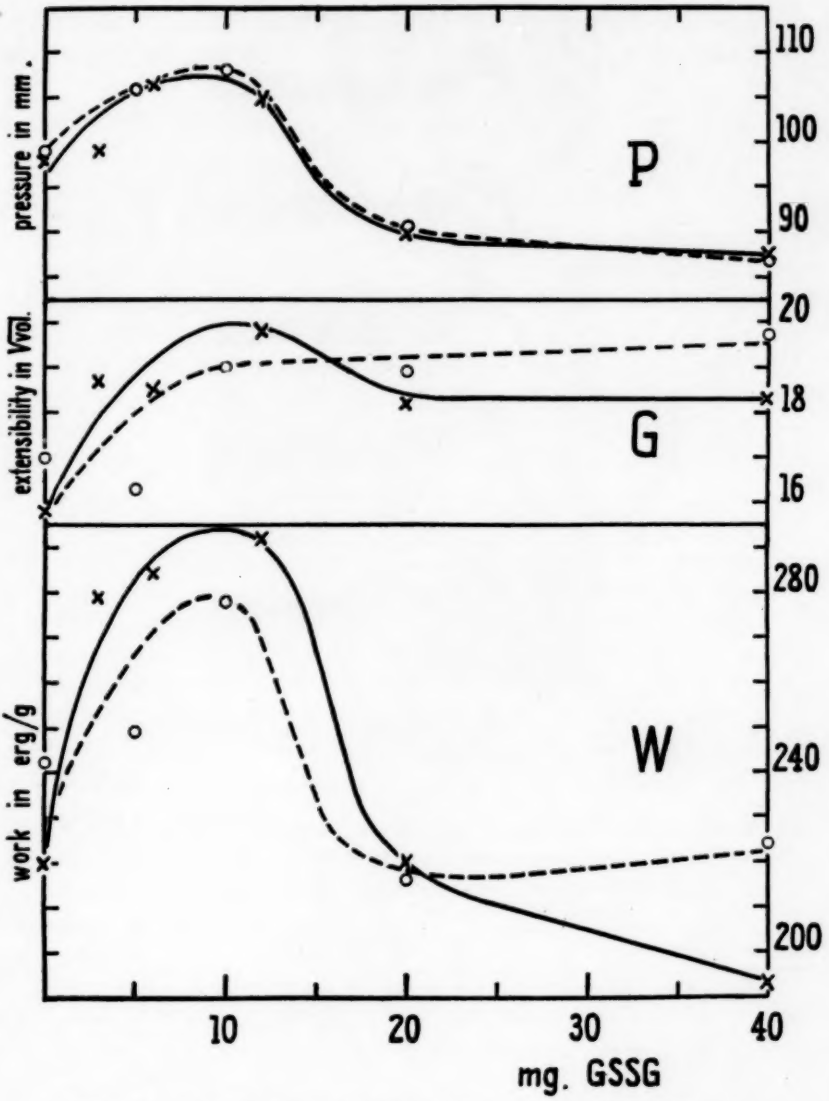

Fig. 2. Results of a duplicate series of extensimeter tests. Doughs as in Figure 1. Mg. GSSG given per 250 g. flour.

the point of maximum swelling power, one of the three maxima of the farinogram mentioned by Bungenberg de Jong (1936). With increasing increments of GSSG the swelling time decreases.

The results of baking tests are given in Table I, where characterizations of the doughs are to be found. These results confirm those found with the extensimeter, namely that GSSG itself had an improving

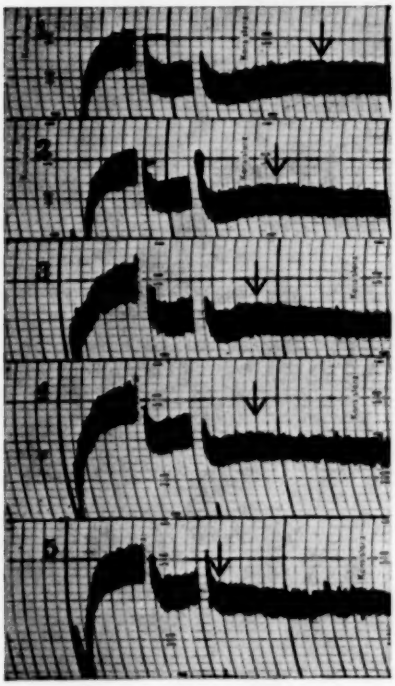

Fig. 3. Farinograms. 300 g. commercial short patent flour, 2% salt, 3% yeast, 56% water. Two rest periods of 60 minutes each. The arrow shows the point of maximum swelling. Amounts of GSSG per 100 g. flour: Curve *1*, 0 mg.; *2*, 2 mg.; *3*, 4 mg.; *4*, 8 mg.; *5*, 16 mg.

TABLE I

THE EFFECT OF THE ADDITION OF GSH, GSSG,[1] AND BROMATE ON LOAF VOLUME

Supplement	Flour	Loaf vol. with supplements (mg.) per 100 g. flour					Max. difference	Remarks on treated doughs
		0	2	5	10	15		
		cc.	*cc.*	*cc.*	*cc.*	*cc.*		
GSH	I	475	—	430	405	420	−14%	Doughs soft and sticky. Texture very poor.
GSSG	II	515	—	535	525	525	+ 4%	Doughs firm, but lack liveliness. Texture good.
$KBrO_3$	II	515	545	—	—	—	+ 6%	Dough lively. Texture good.

[1] Sixty mg. GSH was dissolved in 200 cc. water and oxidized with 19.5 cc. *n*/100 iodine.

effect, which was the most marked for an addition of about 5 mg. GSSG per 100 g. flour. The addition of a larger amount still gives a firm dough, although the latter is not nearly so lively as a bromate-treated dough. The different increments of GSSG did not affect the texture of the finished loaves nor the fermentation time. This was

also confirmed by gassing tests, which showed no difference either in the rate of gas production or in the amount of total gas produced.

Addition per 100 g. flour	Total gas in 5 hours (25 g. flour)
None	264 cc.
2 mg. GSSG	264 cc.
16 mg. GSSG	258 cc.

The oxidation of GSH by iodine is usually explained by the following reaction: $2GSH + 2I = GSSG + 2HI$. The oxidation of 4 mg. GSH will consequently introduce the equivalent of 0.13 cc. $n/10$ HI in a dough. The presence of this amount of acid cannot explain the action attributed to the GSSG. Bailey and Le Vesconte (1924) showed that on increasing the hydrogen-ion concentration (below pH 6.1) the extensibility (G) decreases. On adding oxidized glutathione, however, we have always measured an increase in the factor G (Fig. 2).

Summary

It has been shown that oxidized glutathione influences the swelling power of gluten, but has no action on the gassing power of a dough.

A mechanical dough tester, as well as the baking test, revealed that very small amounts of oxidized glutathione (about 1 part in 25,000) had a beneficial effect on a dough. The improvement of baking strength caused by the addition of bromate is not merely due to the suppression of the harmful effect of the protease activator glutathione, but can partly be explained by the subsequent presence of oxidized glutathione in the dough.

Acknowledgment

The author is indebted to Mr. F. Brassel for assistance with the experimental work.

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DOUGH IMPROVEMENT STUDIES. III. FURTHER STUDIES ON THE OXIDATION OF GLUTATHIONE BY BROMATE, CHLORATE, AND PERSULPHATE

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Waldschmidt-Leitz and Balls (1935) state that the proteases of seeds are mixtures of an aminopolypeptidase, a dipeptidase and a proteinase; the latter can be activated by sulfhydryl compounds (*e.g.* cysteine, glutathione). Balls and Hale (1936) express the opinion that wheat flour proteinases are of the papain type. This idea is also shared by Jørgensen (1939). On the other hand Harris (1939) found that flour proteases do not react like papain, and Read and Haas (1939) that wheat malt proteases do not react like papain.

According to the theory of Jørgensen (1935), in support of which he has published further proof (1938, 1939), oxidizing agents used in baking only suppress the activating action of glutathione on the powerful but normally inactive proteolytic enzymes present in wheat-flour doughs. Sullivan, Howe, and Schmalz (1936) caution against too narrow a view regarding the activation of proteases by sulfhydryl compounds, and Bungenberg de Jong (1939) takes the same attitude regarding the bromate action. Freilich and Frey (1939) have discussed the effect of "excess bromate" in a dough. They state that it is evident from some of their work that the effects of bromate and other oxidants are not confined simply to the inhibition of proteases.

By analogy with other biological oxidations it is conceivable that in the natural oxidation process in wheat flour on "aging," several intermediary steps are involved. In the case of respiration the admirable investigations of Szent-Györgyi (1937) and coworkers have actually fixed the numerous steps in oxidation showing exactly the fate of the hydrogen and of the oxygen. The oxidation proceeds by gradual steps because otherwise much too much energy would be lost. This might well be the case in the natural "aging" of flour, in which process the oxidation of glutathione and the inactivation of protease are probably two of the steps.

Comparing the difference between the action of bromate on the one side and oxygen or inorganic peroxides on the other, Baker and Mize (1937) state that, assuming that the reactions produced by oxidizing agents are slow, this difference can be explained by the ability of bromate to remain active in the dough throughout the entire period of mixing and subsequent fermentation, whereas the other reagents—

oxygen and peroxide—are removed either by aeration of the oxygen from the dough by the escaping carbon dioxide, or the decomposition of the peroxide by catalase into oxygen and its subsequent removal by aeration. It has recently been shown that pure glutathione is indeed only slowly oxidized by one of the most potent improvers used in baking, namely potassium bromate (Ziegler, 1940). The pH did not have any marked effect below pH 6. The same applies to the oxidation of GSH by iodate, periodate, and dehydroascorbic acid, as will be shown later.

Effect of GSH, Alone and in the Presence of Salt

Through the addition of reduced glutathione serious deterioration of gluten suddenly set in after 5 minutes of kneading (Fig. 1, curve

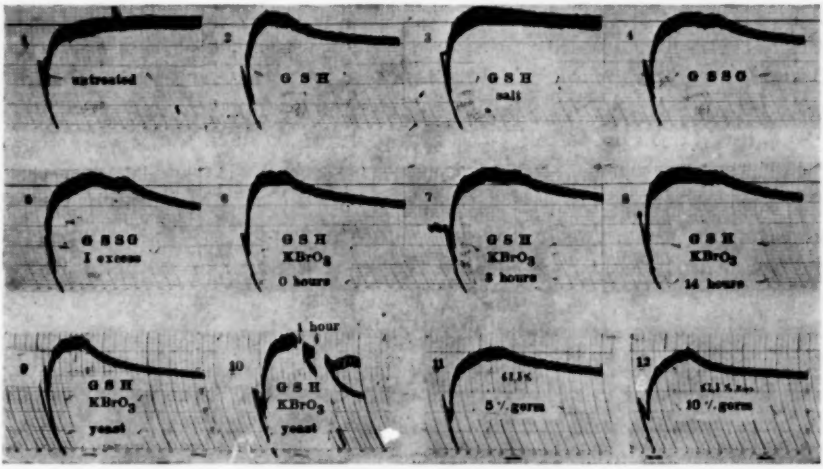

Fig. 1. Farinograms of doughs prepared with 300 g. flour and 180 g. water, without yeast or salt, except where otherwise mentioned. (1) Commercial fresh short patent flour alone. (2) 30 mg. GSH. (3) 30 mg. GSH + 6 g. NaCl. (4) Before addition to dough, 30 mg. GSH oxidized with iodine. (5) Like 4, but with an excess of iodine. (6–8) 30 mg. GSH + 15 mg. bromate allowed to react for 0, 3 and 14 hours. (9) 30 mg. GSH + 15 mg. bromate + 9 g. baker's yeast. (10) Like 9, but wit 2 one hour fermentation periods. The curves of the dough without GSH or $KBrO_3$ have been drawn in. (11) 95% flour + 5% germ. (12) 90% flour + 10% germ.

No. 2). All authors agree that GSH acts with surprising rapidity in a dough. As Freilich and Frey (1939) have emphasized, papain however only reacts slowly. Probably a specific GSH effect, as well as the enzyme activation effect, takes place in dough.

Sodium chloride is known to toughen or coagulate the gluten (Fig. 1, Nos. 2, 3), so much so that Bungenberg de Jong (1933) maintains that the physical properties of dough should be tested in the absence of salt, which otherwise eliminates the characteristic differences between flours. Curve 3 shows that salt in the proportion used in baking

protected the gluten to a certain extent against the action of added GSH. Table I gives results obtained with the Chopin extensimeter.

TABLE I

EFFECT OF SALT ON DOUGHS CONTAINING REDUCED GLUTATHIONE, MEASURED BY THE CHOPIN EXTENSIMETER.

250 g. flour. 130 cc. water. P = tenacity; G = extensibility; W = mechanical work.

Short patent flour	Extensimeter factor	No NaCl	5 g. NaCl
No GSH	P	71	75
	G	18.8	21.4
	W	144	214
25 mg. GSH	P	48.5	53.5
	G	15.3	21.4
	W	61	109

The addition of 0.01% GSH considerably reduced the water-absorbing capacity, as measured by the factor *P*, and at the same time made the dough very short. The addition of 2% of salt tightened up the doughs without and with GSH. From these extensimeter curves we see that 2% salt did not completely protect the gluten from the action of 0.01% GSH.

The percentages of salt and glutathione added to the extensimeter and to the farinograph doughs were the same. Both farinograph curves (Fig. 1, Nos. 1, 3) needed the same amount of water (180 cc.) to reach the consistency line 500. For the extensimeter curves in each case 130 cc. of water was added with the result that the doughs containing GSH were softer. Consequently the heights of the farinogram and extensimeter curves are not measures of the same factor. The claim is made for both machines that the water absorption is measured. The following figures show the results:

	Without NaCl	With NaCl	Dough, due to NaCl
(*a*) Without GSH			
Farinograph consistency "500" (*cc.*)	180	174	Softer
Extensimeter *P* (*mm.*)	71	75	Firmer
(*b*) With GSH			
Farinograph consistency "500" (*cc.*)	180	180	Same
Extensimeter *P* (*mm.*)	71	53.5	Much softer

Oxidation of GSH by Chlorate

Over 20 years ago, when Kohman and his assistants announced one of the most important discoveries in cereal chemistry, namely the improving effect of bromate and iodate, they had noticed that chlorate had no action in a dough. Jørgensen (1935) used this fact as proof for his theory. However at one time sodium chlorate was being used in the baking industry and has been recommended as an oxidizing agent

by Working (1928). It can be shown that the high mobility of a flour-water dough containing some papain and allowed to stand for one hour can be reduced by the presence of bromate, but not of chlorate.

Hoffmann (1912) and Hoffman, Ehrhart, and Schneider (1913) tested the activation of chlorate by osmium tetroxide, a very strong oxidizing agent, whose reduction product, osmium hydrate $Os(OH)_4$, is black. For this reason osmium tetroxide is often resorted to in microscopical work for the detection of unsaturated fatty acids. The aleurone cells and germ are colored black by OsO_4. Hoffmann states that chlorate alone, in neutral or slightly acid solution, is not an oxidizing agent at all. He also mentioned that OsO_4 is an oxygen carrier. In the presence of chlorate a compound chlorate—OsO_4 is formed and this acts as the oxidant, the chlorate then giving off all its oxygen.

Tests were undertaken to see whether, in the presence of OsO_4, chlorate can oxidize glutathione. The same micro-titrimetric method was used as in a former investigation. A 2% solution of OsO_4 as used in microscopy was taken, but it should be mentioned that this solution was no longer quite fresh. The figures in Table II show the results.

TABLE II

OXIDATION OF GLUTATHIONE (GSH) BY CHLORATE

In all cases 1 mg. GSH in 10 cc. distilled water. Room temperature

No.	Substance added		Reaction time	*n*/500 iodine	Degree of oxidation (approx.)
		mg.	*min.*	*cc.*	%
1	None	—	—	1.70	—
2	Potassium bromate	5	0	1.65	0
3	Potassium chlorate	5	0	1.70	0
4	Osmium tetroxide	1.5	0	1.40	18
5	Potassium chlorate + osmium tetroxide	5 1.5	0	0.85	50
6	Potassium bromate	5	60	0.65	60
7	Potassium chlorate	5	60	1.70	0
8	Osmium tetroxide	1.5	60	0.80	50
9	Potassium chlorate + osmium tetroxide	5 1.5	60	0.25	100

Whereas 5 mg. bromate oxidized 1 mg. GSH to the extent of 60% in one hour (Ziegler, 1940), chlorate alone had no action (Table II, No. 6, 7), confirming the well-known fact. OsO_4 alone (No. 8) oxidized GSH about as quickly as bromate did. Chlorate, in the presence of OsO_4, oxidized GSH twice as rapidly as OsO_4 alone and twice as rapidly as the same amount of bromate. The rate of oxidation of GSH was greater by the combination chlorate-OsO_4 than by any one of the two compounds alone. On baking (hand-mixing) the expected improvement did not occur, as the figures in Table III show.

TABLE III

EFFECT OF THE ADDITION OF CHLORATE AND OSMIUM OXIDE

Fermentation time 90 minutes. Experimental flours from Plate wheats.

	Additions in mg. per 100 g. flour				
Chlorate	—	2	2	4	--
OsO_4	—	3	4	3	—
Bromate	—	—	—	—	2
	Loaf volume in cc. per 150 g. dough				
Flour I	480	—	490	—	—
Flour II	470	465	—	455	515

The fermentation rate seemed to be unaffected by the addition, and the dough was somewhat stickier than the untreated dough. It is thought that the OsO_4 is reduced by the unsaturated fatty acids and is consequently not free to act in combination with the chlorate.

Oxidation of GSH by Bromate

A ratio for GSH: $KBrO_3$ = 2 : 1 was chosen because, in a former study, GSH was shown to be only slowly oxidized under this condition, and we wanted to test out whether the rate of oxidation would be increased by the presence of dough constituents (Fig. 1, No. 2, 6–8). The GSH and bromate solutions were allowed to react together for 0, 3, and 14 hours before being used as part of the absorption water in a mixing test. According to previous results roughly 0, 25, and 75% of the GSH could be expected to be oxidized by the amount of bromate used in 0, 3, and 14 hours respectively. The same phenomenon appears as when the oxidation is done by iodine (curve No. 4). After a certain time a definite kind of gluten breakdown occurred. As in our titration tests already published, a progressive oxidation of GSH by bromate took place and it did not appear to be catalyzed by the constituents of a nonfermenting dough.

Curves 6 and 9 (Fig. 1) show that the presence of yeast does not increase the rate of oxidation. Two fermenting doughs without salt were made (curve No. 10), with and without a mixture of GSH and bromate. Another test made with GSH and yeast without fermentation period, gave much the same curve as No. 2. Even after two hours (curve No. 10) the bromate did not stop further injury to the gluten, any more than in a nonfermenting dough, again showing that flour constituents do not appear to increase the rate of oxidation of glutathione by bromate.

Although curves No. 11 and No. 12 were prepared some 4 years ago, they are given here as they show the same type of gluten breakdown, curves No. 8 and No. 11 as well as No. 2 and 12 being very similar.

Sullivan, Howe, and Schmalz (1937) have shown that by heating germ or glutathione under increased pressure the deleterious effect can be inhibited. In view of the work of Harington and Mead (1935) who have shown that glutathione can be easily destroyed by boiling in aqueous solution, yielding glutaminic acid and cystylglycyl anhydride, it is possible that the boiling under increased pressure (which results in a boiling point of water above 100°C.) caused a destruction of the molecule rather than an ordinary oxidation to oxidized glutathione.

Oxidation of GSH by Iodine

Iodine, known to oxidize GSH immediately, was used in a former study to follow the rate of oxidation by bromate (Fig. 1, Nos. 2, 4, 5). Thirty mg. GSH was oxidized with iodine just before the farinograph test was made (curve No. 4). Comparing Nos. 2 and 4, it will be seen that in the latter dough the same type of gluten breakdown as in the GSH dough (No. 2) and in the bromate tests (Nos. 6–8) occurs. With completely oxidized glutathione (No. 4) the breakdown occurred 2 minutes later than in the GSH dough. The oxidized glutathione seems to be reduced again in the nonfermenting dough.[1]

Oxidation of GSH by Persulphate

As Kent-Jones (1939) points out, Plate and English wheat flours are especially responsive to persulphates, which have been in use for many years in Europe. Baking tests show that about three times as much persulphate as bromate is needed to improve flour and that the action is different in each case. According to Read and Haas (1937) Elion and Elion patented a process in 1932 claiming that persulphates increase the saccharogenic activity of flour very appreciably. Flohil (1936) was able to inhibit the injurious effect of malted flour extract on gluten by ammonium persulphate, among other oxidizing agents. Kosmin (1934) showed that this salt can even damage gluten.

Titration tests were carried out to see to what extent persulphate was able to oxidize pure glutathione. Figures in Table IV show that an amount (50 mg.) which would be amply sufficient for oxidizing 1 mg. GSH in 60 minutes by bromate had hardly any action. The following figures taken from Tables II and IV show that it needs more than 30 times as much persulphate to oxidize GSH as fast as bromate does.

One mg. GSH treated with:	Degree of oxidation after 60 minutes
5 mg. bromate	60%
50 mg. persulphate	15%
150 mg. persulphate	40%

[1] Skovholt and Bailey (Cereal Chem. 12: 321–355, 1935) emphasize the rapid increase in reducing sugars during mixing. GSSG can be reduced by maltose.

TABLE IV

EFFECT OF SOME SUBSTANCES ON THE REDUCING POWER OF GLUTATHIONE
In all cases 1 mg. GSH in 10 cc. distilled water. Room temperature.

No.	Substance added		Reaction time	*n*/500 iodine	Degree of oxidation (approx.)
		mg.	*min.*	*cc.*	%
1	Potassium persulphate	50	60	1.45	15
2	Potassium persulphate	150	60	1.00	40
3	Potassium thiocyanate (rhodanide)	5	60	1.75	0
4	Formaldehyde (38%)	2 drops	0	1.70[1]	0
5	Formaldehyde	2 drops	60	1.65	0

[1] Two drops formaldehyde alone needed 0.1 cc. iodine.

Therefore it would seem that although bromate action in baking can be explained to a certain extent by its oxidation of GSH, the improvement by persulphate is mainly due to action on other factors.

Effect of Thiocyanate on GSH

In a study on flour improver action Flohil (1936) reported baking tests which showed that KCNS added in fair quantities is of real value as an improver, although its toxic character makes it absolutely unfit for human consumption. As KCNS also inhibited proteolytic action in his tests it was considered worthwhile to see what effect it might have on glutathione. There was no action on the reducing power of GSH by a small amount of KCNS (Table IV). The improvement is due to the big increase it causes in the swelling power of gluten, according to Bungenberg de Jong (1936), and not to any effect on glutathione.

Effect of Formaldehyde in Dough

In view of the work of Fujita and Numata (1939), who have recently mentioned that formaldehyde can partially destroy the glutathione molecule, titrations were made (Table IV, Nos. 4, 5). It could not be expected that it would oxidize GSH, being a reducing agent itself. No effect was noticed in the titration. Formaldehyde has a powerful action on the coagulation of gluten (Resnitschenko and Popzowa, 1934; Kosmin, 1934).

Although not bearing directly on the problem treated in this paper, it may be interesting to mention the effect of formaldehyde on the gassing power of dough. Here is a case where reliance on a maltose figure alone leads to very wrong conclusions. In the presence of formaldehyde—as was observed in the author's laboratory—the production of reducing sugars is almost normal, as measured by the

usual diastatic activity determinations. In spite of this fact no gas is produced, showing that only the last stages of alcoholic fermentation are affected by the presence of the aldehyde. It could be shown that neither maltose nor glucose are fermented by yeast in the presence of formaldehyde, known as a strong enzyme poison.

Summary

Sodium chloride only partially protected gluten against the direct or indirect action of reduced glutathione.

Chlorate is known to be useless as a bread improver and to exert no action on the oxidation of glutathione by itself. The compound which is formed between chlorate and osmium tetroxide oxidized glutathione more rapidly than osmium tetroxide alone and more rapidly than the same amount of bromate. In a dough, however, no improvement was noticed. This may be due to a reaction of the unsaturated fatty acids of flour with the osmium oxide.

The slow oxidation of glutathione by bromate was followed in a nonfermenting and in a fermenting dough.

Glutathione oxidized by iodine or bromate recovered its deleterious effect in a dough containing neither salt nor yeast after several minutes of mixing, probably due to the production of reducing sugars.

It has been shown that the action of the widely used bread improver persulphate must be mainly due to factors other than the oxidation of glutathione, which does proceed although extremely slowly.

The improvement by the addition of thiocyanate is not connected with any action on glutathione. It is known to have a powerful swelling effect on gluten.

The height of the farinogram and of the Chopin extensimeter curve did not measure the same factor, although both are said to be a measure of water absorption.

Acknowledgment

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RUSSIAN EXPORT WHEATS—COMPOSITION AND CHARACTER OF THE 1938 CROP

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(Received for publication April 27, 1940)

Russia has always been one of the larger wheat-producing countries of the world. From 1909 to 1913 Russia accounted for 19.5% of the world total production, and in the period 1928 to 1932 she produced 17.1% of the total. The exports of Russian wheats for these years were as follows:

TABLE I

RUSSIAN WHEAT EXPORTS, 1910–13 AND 1930–36

Year	Bushels: 000 omitted	Year	Bushels: 000 omitted
1910	224,986	1932	7,764
1911	142,806	1933	27,435
1912	96,689	1934	20,199
1913	122,063	1935	91,633
1930	1,312	1936	92,800
1931	26,369	—	—

Holland imported from Russia the following quantities of wheat in recent years:

TABLE II

HOLLAND IMPORTATIONS FROM RUSSIA, 1930–39

Year	Bushels: 000 omitted	Year	Bushels: 000 omitted
1930	4,620	1935	904
1931	11,566	1936	808
1932	3,768	1937	1,480
1933	2,834	1938	4,540
1934	661	1939	1,592

Of the 1938 Russian crop the Netherlands again had a share, and 5,498,000 bushels were imported. The experiences obtained with this crop when technically viewed are very interesting. As to the part of the country where the wheat was grown little is known, but the wheat was shipped from South Russian ports and was probably spring wheat. The various lots were denoted by number, but it was impossible to draw any conclusion from such numbers which might indicate some particular quality or grade. For example in 1937 No. 441/0 was a wheat yielding flour of good baking value, whereas in 1938 a wheat of that number was quite unsuitable for baking purposes.

In this country during the last few years it has been compulsory to

use a relatively high percentage of home-grown wheat in the mill mixture. This has been as high as 35%, millers being at liberty to use for the remaining 65% any wheats available, and these have consisted of wheats from Canada, United States of America (hard winter wheats), Argentina, Russia, and Rumania.

As the numbers under which Russian wheats were sold bore no relation to their respective qualities, it was necessary, in order to avoid trouble, to examine every parcel. The following summarized results of analytical tests show how necessary it was to carry out proper tests before working these Russian wheats into the mixture. Incidentally, it is of interest to record that all the shipments referred to were essentially red or brown-red varieties, vitreous and fairly hard, and included no soft white wheats. The shipments were all, on the whole, fairly clean; any admixture of weed seeds etc. was not more than 0.5%.

In the table which follows the gluten-content figures, wet and dry, refer to the flour.

TABLE III

CHARACTERISTICS OF RUSSIAN EXPORT WHEAT

Wheat No.	Test weight	Weight per 1000 kernels	Moisture content	Protein (on dry substance)	Gluten	
					Wet	Dry
	Lbs. per bu.	*g.*	%	%	%	%
*127 R	64.00	29.5	11.5	12.26	31.0	9.4
*139	64.00	29	11.5	12.46	31.0	9.8
†402/3	63.28	30	11.0	13.49	30.6	9.5
*402/4	63.60	27	11.0	13.48	30	9.5
†402/5	63.12	28.5	11.0	12.07	22.6	7.0
*421/2	65.54	31	10.5	13.55	33.9	10.1
*421/3	65.08	31	12.6	13.98	33.2	10.5
*421/32	64.00	32	12.8	—	37.2	11.3
*421/61	67.68	32.5	9.4	13.78	34.2	10.5
†431/3	64.88	24	11.1	12.95	29.5	8.9
*431/4	65.80	23.5	11.1	12.47	28.4	8.7
*431/61	66.24	24.5	10.6	14.03	34.6	11.8
‡441/0	62.68	—	10.8	12.95	Not washable	
*441/2	65.16	33.0	10.4	—	31.3	9.5
*441/4	64.44	26.7	11.4	11.4	32.0	10.1
†441/5	63.28	—	10.9	—	36.3	10.5
‡441/6	62.88	31.0	10.0	13.9	Not washable	
†441/53	64.00	29.0	12.1	11.44	22.1	7.1

* Slightly affected by the wheat bug. † Heavily affected. ‡ Very heavily affected.

Damage to the Grain due to Wheat Bug Infection

Almost every wheat sample showed damage caused by the wheat bug. The degree of damage ranged from only lightly affected to badly damaged, in fact samples No. 441/0 and No. 441/6 of the 1938 crop were so heavily infected that it was impossible to bake a good loaf

from the flours of these samples; furthermore it was not possible to retain any of the gluten in a washing test (see Table III). Other lots badly infected were No. 402/3, No. 402/5, No. 431/3, No. 441/5 and No. 441/53.

Wheat bug infection was described for the first time by Berliner.[1] Prior to this other investigators had noted and recorded the occurrence of exceptionally bad gluten in some wheats, but were not aware that this insect was responsible for the damage.

The wheat bug makes its attacks by forcing an entry with its proboscis into the berry when it is in the milk stage or when the grain is green, and by this means it extracts the contents of the berry. Apparently at the same time it deposits a matter very rich in proteolytic enzymes. On ripe kernels the damage by the wheat bug is indicated by a pale yellow speck or mark, with a small dark point, and the kernels are often shrivelled. The small spot indicates the place where the bug has pierced the berry.

Method of Test Milling the Wheat Samples

After the usual preliminary preparation and cleaning, all samples were ground on an experimental mill to produce a straight-run flour of about 15% moisture and 0.54%–0.58% ash, the latter calculated on dry matter.

The results of the determinations of gluten are given in Table III. It was impossible to wash out the glutens of the very heavily infected samples No. 441/0 and No. 441/6; during the washing out of these samples no gluten whatever was recoverable, and with several other samples it was only possible to recover gluten with difficulty.

Kneading Tests (Farinograph)

In view of the foregoing it was only to be expected that there would be large differences among the various samples, and curves typical of such differences are given in Figure 1.

In the case of curves (farinograms) No. 441/0 and No. 441/6 (those samples yielding no gluten in the gluten washing test), it will be seen that after a kneading time of three to four minutes, in which the doughs attain a maximum consistency of 500 g., a sharp decrease in consistency is apparent. After a kneading time of ten minutes the drop in the consistency is 210 g. and 280 g. respectively. The condition of the dough at this point is sticky.

In contrast to the poor results on these inferior samples are the curves of samples No. 421/3 and No. 441/4, which are normal and indicate quite good and sound characteristics. Other curves range between the above two extremes.

[1] E. Berliner: "Leimkleberweizen" ist "Wanzenweizen," Mühlenlab. No. 4 (Mühle, Heft 32), 1931.

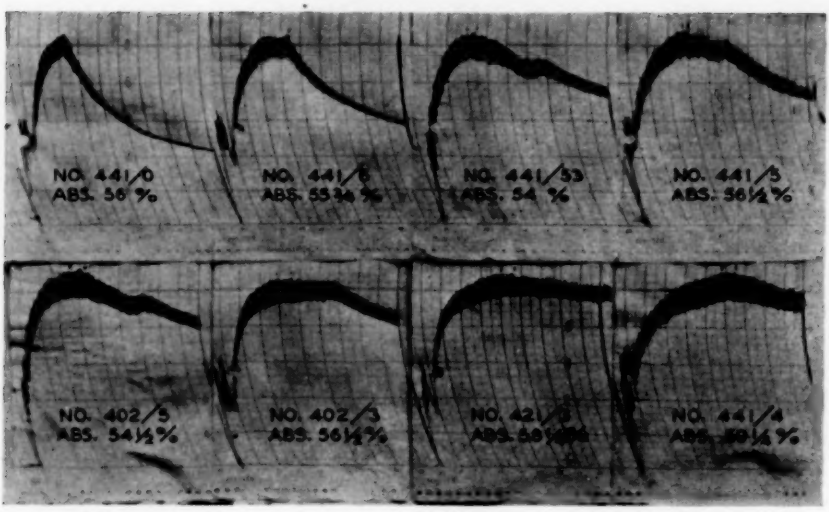

Fig. 1. Farinograph curves.

The kneading test has the advantage that it permits flours of even very inferior quality to be evaluated, and as these are made into dough in a mechanical dough mixer the examination can be made without the necessity of touching the dough with instruments or with the hand.

Extensibility Tests

These tests, when using one or other of the well known testing devices, for example the Chopin Alveograph, the Brabender Extensograph, etc., are in contrast with the kneading tests, in as much as the doughs have to be taken out of the dough mixer and manipulated by hand for weighing and shaping the dough before the extensibility test can be made.

Thus it is very difficult, when making the test for extensibility, to handle and examine inferior flour doughs because of their stickiness. Consequently Nos. 441/0, 441/5, 441/6 (in which it was impossible or difficult to retain any gluten in the washing test) could not be so tested. Obviously this is an undesirable feature and is in itself indicative of very poor quality.

Other samples could be measured only after a dough rest of 45 minutes at a temperature of 27° C., but this was no longer possible after a period of 135 minutes. Samples falling into this category were Nos. 127R, 139, 402/3, 402/4, 402/5, 441/53, 431/4. Some of the curves are shown in Figure 2.

The following remarks apply to all the samples to which it was possible to apply the extensibility test either partly or completely.

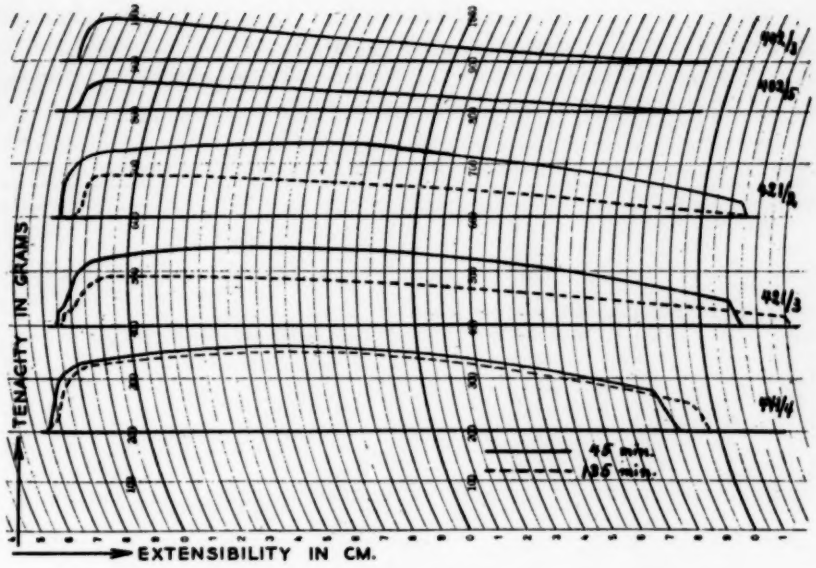

Fig. 2. Tenacity and extensibility of doughs (53% water on 15% basis).

The extensibility of the dough is poor, for the dough is very short. The elasticity leaves much to be desired, and this is shown by the abnormal diagram curves (see Figure 2, Nos. 402/3 and 402/5). The tenacity (resistance to extension) is often slight initially, and there is a big difference in tenacity between a dough rest of 45 minutes and a dough rest of 135 minutes at 27° C. (see curves for Nos. 421/2 and 421/3). Again it will be observed that sample No. 441/4 is foremost in quality.

Baking Tests

The baking tests were carried out using water with 2% of salt and 2½% of yeast, the temperature of the dough being 27° C. and the water absorption calculated on a moisture content of 15% in the untreated flour. All flour samples were baked: (*a*) untreated, (*b*) treated with potassium bromate. Because of the treatment with potassium bromate the water absorption was increased by 1½% and in most cases an improvement in volume resulted. The poorer samples however were an exception and responded only slightly or not at all to treatment.

The results of the baking tests confirm the results of mechanical dough examination by kneading and extensibility tests. The wheats No. 402/3 and No. 402/5 were classified as very poor, while Nos. 441/0, 441/5, 441/6, 441/53 were classified as unsuitable for baking purposes.

TABLE IV

BAKING TESTS

Wheat No.	Bread volume, 100 g. flour (untreated)	Bread volume, 100 g. flour (treated)	Water absorption	Remarks
	cc.	*cc.*	%	
127 R	507	657	56¼	
139	473	621	56¼	
402/3	378	588	56½	(Dough weakened markedly, resultant loaf flat)
402/4	468	650	56½	
402/5	384	574	54½	(Dough weakened markedly)
421/2	532	667	56¾	
421/3	505	748	58¼	
421/32	625	701	56½	
421/61	575	704	58¾	
431/3	384	627	54½	
431/4	413	668	54¾	
431/61	420	706	55¾	
441/0	290	297	56	(Dough exceptionally bad, very slimy; loaf collapsed)
441/2	450	633	56¼	
441/4	606	672	56¾	
441/5	364	451	56½	(Dough very bad, weakened markedly; loaf flat)
441/6	326	329	55¾	(Dough and loaf as 441/0, collapsed)
441/53	332	483	54	(Dough very bad, weakened markedly; loaf rather flat)

Behavior of Russian Wheats when Used in a Blend

Normally when sound wheats are blended, the influence of any one kind on the quality of the grist as a whole is more or less in direct proportion to its own inherent qualities, and to the quantity used.

With Russian wheats affected by the wheat bug this was not the case, owing to the fact that the incorporation of a percentage of this infected wheat in the blend introduced an abnormally high percentage of proteolytic enzymes, whose destructive effects were very prominent in the flour and dough. Actually the presence of such Russian wheat in the grist affects the blend in two ways: (*a*) by the poor quality of its gluten, and (*b*) by its excess of proteolytic enzymes. The unfavorable effect was not confined in the grist to the Russian wheat itself but was transmitted to the other wheats in the blend, by virtue of its high protease content.

An investigation was made of the effect of introducing a percentage of No. 441/0 (gluten nonretainable in washing test) into the wheat mixture. This was carried out by adding flour milled from No. 441/0 to a straight-run control flour which had been milled from a wheat mixture normally used in Holland and consisting of Manitobas

(Canada), hard winter (U. S. A.), Plate (Argentina), and a percentage of home-grown (Netherlands) wheat, the ash content of this flour being 0.58%, calculated on dry matter. The resulting blended flour was examined mechanically by kneading and extensibility tests, using the Extensograph (Brabender) and the Comparator (Buhler).

The extensibility tests especially showed that an admixture of as little as 2% of No. 441/0 exerted a distinctly unfavourable influence on the quality of the flour.

TABLE V

EXTENSIBILITY TESTS WITH EXTENSOGRAPH AFTER DOUGH RESTS OF 45 AND 135 MINUTES AT 27°C.

(Dough was made using 53% water and 1.65% salt)

Russian wheat flour 441/0, admixed	Dough rest of 45 minutes		Dough rest of 135 minutes		Difference in tenacity at 45 and 135 min.
	Tenacity	Extensibility	Tenacity	Extensibility	
	g.	*cm.*	*g.*	*cm.*	*g.*
0%	250	23½	250	21½	0
2%	250	22	235	21	15
4%	210	23½	180	23½	30
6%	205	23½	150	22½	55

The addition of flour milled from the infected Russian wheat obviously caused the tenacity of the flour to decrease; without any addition of such flour the tenacity of the control flour after dough rests of 45 minutes and 135 minutes was the same. With a small percentage of the affected flour added thereto (2%) the tenacity of the dough not only decreased after 45 minutes but to an even greater extent at 135 minutes. The drop in tenacity between 45-minute and 135-minute periods was not in proportion to the percentage of affected flour used, but such drop was in increasing proportion, for example:

A 2% admixture resulted in a drop in tenacity of 15 g. on 250 g. = 6%.
A 4% admixture resulted in a drop in tenacity of 30 g. on 210 g. = 14%.
A 6% admixture resulted in a drop in tenacity of 55 g. on 205 g. = 26%.

The results given in Table V were confirmed by extensibility tests made on the Comparator (Buhler). Repeat tests on the extensograph, this time with yeast doughs using 2½% of yeast, yielded similar and confirmatory results. Baking tests gave indications substantiating the mechanical tests given in detail above.

To complete the investigation a further series of tests was made by adding proportions of the affected Russian wheat flour to soft flours milled from home-grown wheat, and the tests in this series gave results similar to those of the tests made with the normal baker's grade of flour.

Summary

The Russian wheat of the 1938 crop imported into the Netherlands was carefully examined. All parcels of Russian wheat of this crop were more or less damaged by the wheat bug. The protein determination of such wheats does not allow satisfactory conclusions as to the true baking value of these wheats.

In view of the foregoing it was necessary to grind every parcel of the wheat experimentally before incorporating such wheat into the wheat blend. Straight-run flour was therefore obtained on a test-milling plant, this flour having an ash content of 0.55%–0.58% and each of the flours so milled was subjected to mechanical examination of the flour followed by routine test bakings. Of the samples examined 25% were so badly affected that they were unsuitable for baking purposes. The remainder varied between moderate and fairly good in quality.

Extensibility tests of the doughs showed generally the lack of elasticity, and led to abnormal "curves" in mechanical tests. The extensibility of the dough was of moderate character, the doughs being rather on the short side.

The influence of badly affected Russian wheat flour on a good straight-run control flour was examined. It was found that an admixture of only 2% caused a measurable depreciation in the quality of the dough. The extensibility test was found to be extraordinarily sensitive and therefore very suitable for examinations of flours of this kind.

Of particular importance was the fact that the affected Russian wheat flour which was admixed in relatively small proportion was found to increase the proteolytic activity in the mixture out of proportion to what was anticipated, and owing to this the sound flour was badly affected, not in direct proportion to the amount added but in progressively increasing proportion. This was reflected in terms of decreasing tenacity of the dough when the resting time of the dough was increased.

THE IMPORTANCE OF GAS-PRODUCTION AND GAS-RETENTION MEASUREMENTS DURING THE FERMENTATION OF DOUGH

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(Read at the Annual Meeting, May 1940)

The Report of the 1938–39 Committee on Methods of Analysis of the American Association of Cereal Chemists (Sandstedt, 1940) comprises among others the following recommendations: (a) that the study of methods for evaluating yeasts be continued, and (b) that the investigation of gas retention be continued.

Although numerous analyses and separate determinations have been proposed for the evaluation of flour strength and dough properties, an increasing number of cereal chemists believe with good reason that gas production and gas retention during the fermentation of dough are the most important factors of bread production. The writer has been astonished to find that the correctness of this opinion does not seem to be sufficiently recognized, and a short historical review of the literature, therefore, may support this thesis.

Nearly half a century ago, H. Elion (1893) drew attention to the fact that in the fermentation of dough different factors have such an influence on the dough volume that the quantity of gas produced becomes a factor of minor importance, and he pointed out that various other flour properties influence the rising of the dough during fermentation and in the oven.

As far as I am aware, Maurizio (1902) was the first to publish extensive experiments which made a clear differentiation between gas-production and gas-retention capacities. Wood (1907), using the device published previously by H. Elion (1903) (see E. Elion, 1933), confirmed Maurizio's statements.

Various definitions of flour strength (Jago, 1895; Humphries and Biffen, 1907; Wood, 1907; Humphries, 1907; Humphries and Simpson, 1909) also lead to a clear differentiation between gas production and gas retention and to suggestions that these factors be considered as components of strength.

Numerous papers dealing with the importance of gas production and gas retention have appeared during the last three decennia. I refer only to Bailey (1916), Bailey and Weigley (1922), Bailey and Johnson (1924), Blish and Sandstedt (1927), Working (1929), St. John and Bailey (1929), Fisher and Halton (1929, 1929a), Jørgensen (1931), Markley and Bailey (1932), Blish and Hughes (1932), Geddes and

Larmour (1933), Larmour and Brockington (1934), Kosmin (1936, 1937), Clark (1938), Kent-Jones (1937, 1939), and Bailey (1939).

From these papers it is evident that gas production and gas retention are the two factors that predominate in determining the character of the bread. Brabender (1933, 1934) stressed the importance of *properly balanced* gas-production and gas-retention capacities. He pointed out that dough development and gas production both reach a maximum at certain moments during the fermentation of the dough, and that in order to obtain the best baking results, the moments when these maxima are reached should coincide. As a matter of fact, good gas-production and gas-retention capacities are indispensable for good bread, but even with both the resulting loaf may be unsatisfactory, if the dough development starts too late or too early with regard to the available gas production. A good gas production during the fermentation of the dough must, therefore, be accompanied by a good gas-retention capacity during the period when the gas production is available.

From the foregoing one may conclude that the measurement of both gas production and gas retention *during the whole course of fermentation* must be considered as extremely important, as well for the flour miller as for the baker.

Whereas several devices for the measurement of gas production have long been available, this is not the case for gas retention, and this may be one of the reasons that a number of the studies mentioned above have been made with baking tests, in which the personal factor plays an important role. Markley and Bailey (1932), discussing previous nonautomatic methods for the determination of the relation between the production of gas in and loss of gas from a fermenting dough, state that these methods are laborious, requiring practically the constant attention of the technician, and they propose an automatic gas-production registering mechanism, the saving in time of the operator justifying the added cost of the entire device.

In recent years several recording apparatuses for flour research have been constructed in order to eliminate the personal factor involved with baking tests (see E. Elion, 1939). As far as gas production is concerned Brabender's fermentograph has claimed attention. Schmalz and Sullivan (1938), however, pointed out some errors involved in the measurement of gassing power by this device, and stated that several errors can be overcome and compensated for, except a loss of gas by diffusion through the rubber balloon, which, according to these authors, is too large an error to be disregarded.

Another recording device for gas production is the S. I. A. Fermentation Recorder. It is said that this instrument, although not designed for it, can be modified to measure gas retention too. The au-

thor does not have access to any published data obtained with this apparatus.

The complexity of the problem of gas retention may be one of the reasons for the lack of recording devices for its measurement. As a matter of fact, in efforts to approach the problem from other sides, several instruments have been developed recording only separate dough properties during short periods of the fermentation. Such separate dough properties, however, are but more or less related to baking quality and the interpretation of the experimental results and their correlation with practical baking may offer difficulties. As a general objection to all devices which record only one single dough property during a short period of the fermentation, such as various machines for physical tests of flour dough, I quote Munz and Brabender (1940), who recently stated: "Dough properties change steadily with time and as a function of mixing, fermentation, and other treatments. It appears important, therefore, to study the rate and direction of these changes rather than to measure dough properties at one stage only." Furthermore, such machines have been designed only for the study of one dough property, and consequently they may fail to demonstrate other very important properties, for the determination of which one must use other recording devices if a more complete picture of the dough properties is to be obtained.

G. Mueller (1935) discusses a number of methods which have been recommended for the determination of separate properties, such as gluten quality, gluten quantity, and extensibility of dough, and he also points out the errors connected with determinations of single properties of this kind.

As stated above, the two essential factors that predominate in determining flour quality and the character of bread are gas production and gas retention. It is obvious that very useful information can be obtained by a device that records exactly these two properties and therefore can replace several other devices recording different individual properties respectively, which together contribute to gas-production and gas-retention capacity. An apparatus which complies with these requirements, *i.e.* which records automatically both gas production and gas retention during the whole course of fermentation, is actually available under the name Chefaro Balance. This device, which has found increasing application in European countries, has been developed by the Chemische Fabriek Rotterdam, of Rotterdam, Holland, and has been described by E. Elion (1939, 1940). It consists of two special precision balances, by which the quantities of gas actually produced by or retained in the fermenting dough are accurately weighed and automatically recorded during the whole course of the fermentation.

Sources of errors connected with other methods have been eliminated and many advantages are present.

Carbon dioxide is collected in a special gas-jar, which is absolutely gas-tight, so that no gas can escape. The dough is surrounded by air

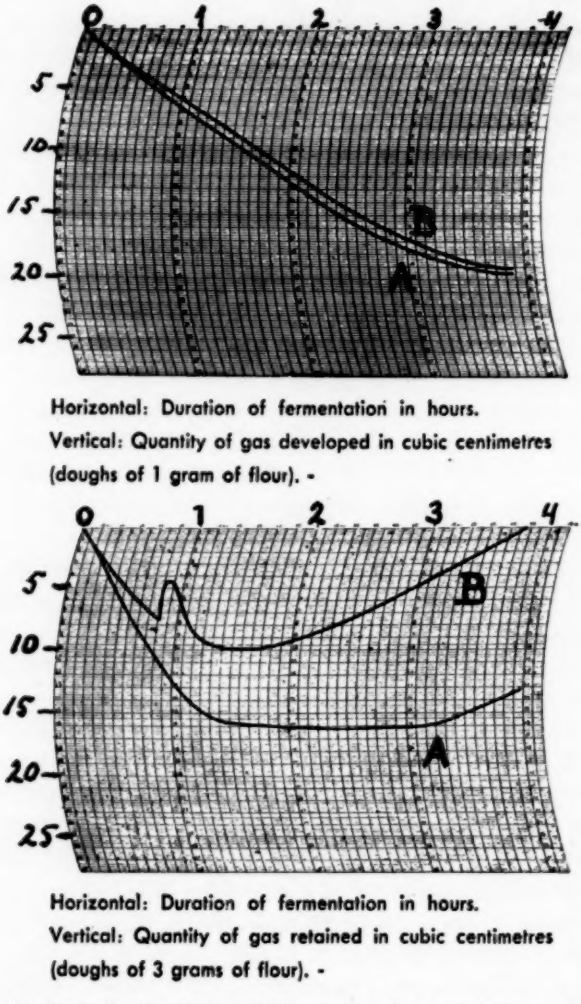

Fig. 1. Gas-production and gas-retention curves as indicators of flour quality.

during the fermentation, as in the bakery, and no materials such as rubber are used. The apparatus records automatically the real gas production and gas retention, *i.e.* the combined effect of numerous known and unknown separate properties, instead of some arbitrary single properties only. The fermenting dough is permanently visible. The time re-

quired to start an experiment is short. The device works automatically and does not require further attention or readings. For large-scale operations several balances can easily be operated by one person. The automatic recording has proved to be a saver of time for the operator. The Chefaro Balance does not require a skilled person for its operation, because faulty determinations can hardly occur. After some little practice excellent duplicate results can be obtained. Figure 1 demonstrates the importance of obtaining both gas-production and gas-retention curves.

There is hardly any difference between the gas production of flours *A* and *B*, both of which produce about 19 cubic centimeters of fer-

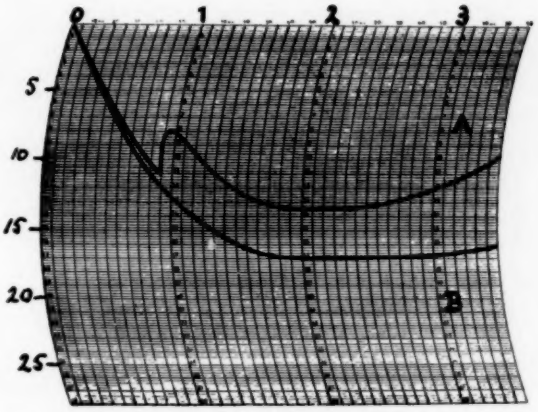

Fig. 2. Gas-retention curves showing effect of flour improvers.

mentation gas (from one gram of flour). In each case fermentation lasts about three hours and a half. Nevertheless it appears that the quality of the two flours differs greatly. Flour *A* gives a loaf volume of 1160 cc., whereas flour *B* only attains a volume of 790 cc.

When examining the gas *retention* capacities, the difference in baking value is soon explained, because a dough made from flour *A* is able to retain 16.2 cc. of fermentation gas as compared with only 10.2 for flour *B*. Moreover the stability (or fermentation tolerance) of flour *A* is much better, the maximum gas-retaining capacities of the doughs *A* and *B* being maintained for 80 and 25 minutes respectively. The gas-production and gas-retention capacities of flour *B* are not properly balanced, as is proved by the temporary escape of gas after 50 minutes.

A further considerable advantage of the Chefaro Balance, which is missing in many of the devices recording separate physical dough properties, consists in the fact that it records the influence of flour improvers and baking agents on gas production and on gas retention (Fig. 2).

An untreated flour (curve *A*) has a maximum gas-retaining capacity of 13.3 cc. A treatment by 12 parts of flour improver to every 100,000 parts of flour strengthens the gluten to such an extent that 17.1 cc. of gas are retained (curve *B*), representing an increase of 28.5%.

The curves show furthermore that the dough stability is much improved by the treatment. The strengthened dough supports the gas pressure longer and therefore may be expected to show better resistance to bakeshop handling.

Since the Chefaro Balance records the influence of flour improvers, this instrument is especially competent to determine the quantity of any improver that will give the best results with a given flour (Fig. 3).

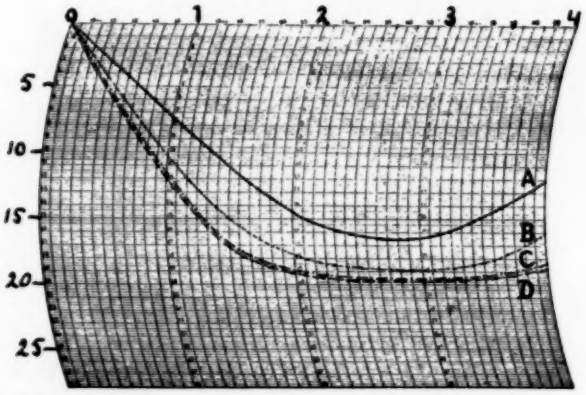

Fig. 3. Variations in gas retention with varying concentrations of improver.
Curve *A* = untreated flour.
Curve *B* = treated with 10 parts of improver to 100,000 parts of flour.
Curve *C* = treated with 12 parts of improver to 100,000 parts of flour.
Curve *D* = treated with 15 parts of improver to 100,000 parts of flour.

The duration of fermentation being recorded on the horizontal axis, it may be observed that the dough when so treated attains its full development more rapidly and that the maximum expansion is maintained for a much longer period.

The gas retention is recorded in a vertical direction and this capacity is increased from 16.5 cc. to 19.7 cc., *i.e.* 3.2 cc. or 19.4%. Further, the graphs show that the treatment with 12 parts of improver is most suitable, the small differences between the graphs *C* and *D* not justifying an increase of 25% in the cost of treatment.

Numerous other applications can be made of the Chefaro Balance, for instance, in studying the influence of the maturing effect of flour storage, different dough mixing methods and different mixing speeds, different yeasts, etc.

When considering the number of published reports of studies on

gas production and gas retention, it may be concluded that the knowledge of these two important factors in bread making could have advanced more rapidly if the Chefaro Balance had been available earlier; and in line with the recommendations of the 1938–39 Committee on Methods of Analysis of the A. A. C. C., mentioned above, the author believes that the application of this new device may prove to be very useful.

Summary

The author cites numerous publications which demonstrate that gas production and gas retention are now generally recognized as the two essential factors that predominate in determining flour quality and the character of bread. He points out that methods which determine only separate properties belonging to the complex problem of gas retention may be of questionable value in the study of flour properties and he stresses the logic and desirability of recording automatically the actual gas production and gas retention during the whole period of dough fermentation, as has been made practically possible by the development of the Chefaro Balance. This instrument consists of two special precision balances, by which the quantities of gas actually produced by or retained in the fermenting dough are accurately weighed and automatically recorded during the whole course of fermentation.

In consequence of some of the advantages and applications of this new device, its further use in the study of gas production and gas retention is recommended.

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THE USE OF HAND-OPERATED SHEETING ROLLS IN TEST BAKING[1]

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(Read at the Annual Meeting, May 1939)

The entire or partial mechanization of the experimental baking test has occupied the interest of cereal technologists for a considerable period of time, and a rather extensive literature pertaining to this subject already exists. A recent paper by Heald (1939) reviews most of the important contributions relative to this subject. The use of sheeting rolls in connection with dough molding has been specifically discussed by Merritt, Blish, and Sandstedt (1932), Geddes and Sibbitt (1933), and Heald (1939). However, the need for additional information and discussion is necessitated by the fact that there is now available, at reasonable cost, a regularly manufactured sheeting roll which is coming into more general use than any previous similar piece of equipment. It is of interest therefore to determine the suitability of this particular type of sheeting roll for use in conducting the baking test.

Experimental

The information presented in this paper deals with the use of the hand-operated sheeting roll manufactured by The National Manufacturing Company, Lincoln, Nebraska. In the study here reported, the rolls were used to sheet the dough previous to molding and were not used during the punching procedure. The method of molding involved removing the dough from the fermentation bowl with the hands slightly greased and placing it on a canvas-covered board. The dough was gently flattened and slightly elongated before being put through the sheeting rolls. The scanty film of grease which the dough collects from the hands and canvas prevents its sticking to the roll surface without the need of greasing the roll or supplying dusting flour.

[1] Subcommittee report, 1938–39 Committee on Standardization of Laboratory Baking.

In order to obtain the desired dough sheet without causing a tearing of the gluten strands, the strong, hard-wheat doughs were put through the sheeter twice: once with a roll spacing of 5/16 inch and again with a setting of 7/32 inch. The pliable soft-wheat doughs were run through the sheeter only once, with the former setting. The sheeted doughs were placed in a wooden trough, of the same width as the bottom of the baking pan, and rolled up fairly tight. The trough prevents the doughs from becoming elongated during the molding process, eliminates the necessity for sealing the dough ends, and allows the operator to introduce easily the doughs into the baking pans. The high pan type was used in this study.

Data for the statistical analysis of the variability of loaf volumes and bread scores between bread molded by hand, in the manner prescribed in the A. A. C. C. Book of Methods (1935), as contrasted with the bread produced by the procedure outlined above, were obtained by making a series of bakes using both a hard and a soft wheat flour. Sixty-five bakes using both methods, with all other conditions identical, were secured using the hard wheat flour; and fifty comparisons were made using the soft wheat flour.

Results

An inspection of Table I indicates that the standard deviations of the loaf volumes for the hard wheat flour were practically identical. A

TABLE I

VARIABILITY OF LOAF VOLUME

Series	Molding method	Type of flour	No. of variates	Mean loaf volume	Standard deviation	Coefficient of variation
A	Hand	Hard	65	665	22.2	3.3
B	S-Rolls	Hard	65	656	22.6	3.4
C	Hand	Soft	50	444	17.3	3.9
D	S-Rolls	Soft	50	442	10.8	2.4

value of 22.2 was obtained by the hand method and a value of 22.6 when the sheeting rolls were used. The use of sheeting rolls on the soft wheat flour reduced the standard deviation from 17.3 to 10.8. The loaf volumes were slightly larger for both flours when molded by hand.

All the bread was scored by assigning numerical values to the important external and internal characteristics of the loaves, including the volume.

In Table II is recorded the variability of the bread scores for both flour types. The use of sheeting rolls does not offer any outstanding

TABLE II

VARIABILITY OF BREAD SCORE

Series	Molding method	Type of flour	No. of variates	Mean loaf score	Standard deviation	Coefficient of variation
A	Hand	Hard	65	93.8	1.6	1.7
B	S-Rolls	Hard	65	94.7	1.5	1.6
C	Hand	Soft	50	82.7	1.2	1.5
D	S-Rolls	Soft	50	83.3	1.1	1.3

advantages from the standpoint of variability in the replication of results but they do give a better uniformity of grain and texture than did loaves molded by hand. This fact accounts for the higher average bread score when the sheeting rolls were employed.

Summary and Conclusions

A series of bakes were conducted, using a hard and a soft wheat flour, in which alternate doughs were handled with and without the use of sheeting rolls during the molding process. When both operations were conducted in the same laboratory by experienced operators, sheeting rolls did not offer any outstanding advantages although they did tend to reduce the variability in the replication of results.

The use of sheeting rolls slightly reduced the loaf volumes and increased the bread scores. This latter result is due to better uniformity of grain and texture.

Sheeting rolls certainly merit careful consideration as an additional step toward the complete standardization of the A. A. C. C. baking test for bread.

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THE AUTOLYTIC DIGESTION OF FLOUR IN RELATION TO VARIETY AND ENVIRONMENT

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Factors which influence the supply of gas in a dough are of great interest and importance to the wheat miller and baker. It is proposed, in this paper, to discuss and produce evidence concerning the effect of variety of wheat and environment during growth on the potential gas production of Australian wheat flour. Since it is not always expedient to bake a sample of flour to evaluate its gassing possibilities, other tests such as can be carried out with small portions of dough or water suspensions of flour are substituted. The latter and most common of the methods employed involves the determination of the sugar formed during an autolytic digestion of ten grams of flour at a given temperature for one hour (American Association of Cereal Chemists, 1935; Kent-Jones, 1927). The result is referred to as either the "diastatic activity" or "maltose figure" and is expressed as milligrams of maltose per ten grams of flour or as percentage of maltose formed. The emphasis in this paper is transferred from the amount of gas which could be produced, to the material from which it originates, namely the sugar, maltose.

The maltose produced during the autolytic digestion of a flour has been shown to be influenced by the variety of wheat from which the flour was made (West, 1932; Markley and Bailey, 1934; Swanson, 1935; Mangels, 1936; Hickinbotham, 1936a, 1936b). The environment of the wheat during growth and harvesting (Bailey, 1925; Mangels, 1926, 1934; Markley and Bailey, 1934; Swanson, 1936; Hickinbotham, 1936b; Farquhar, 1938; Breakwell, 1938; Breakwell and Hutton, 1939) and the milling treatment have also been found to exert an influence on the maltose figure (Johnson, 1930; Pascoe, Gortner, and Sherwood, 1930; Karacsonyi and Bailey, 1930; Markley and Bailey, 1934; Swanson, 1935; Gründer, 1935; Geddes and Aitken, 1935; Hickinbotham, 1936b; Leatherock, McGhee, and Giertz, 1937; Pulkki, 1938; Stamberg and Bailey, 1939a).

Since there are two factors, substrate and enzyme, concerned in the maltose production as determined by an autolytic digestion, it is of interest to know whether the influences of variety, environment, and milling are mainly on the substrate, on the enzyme, or on both.

Variation in the enzyme factor between different flours may be a result of difference in amounts and/or kinds of amylase. At present

two or probably three amylolytic enzymes capable of attacking uncooked starch are considered to be present in wheat flour (Sandstedt, Blish, Mecham, and Bode, 1937; Blish, Sandstedt, and Mecham, 1937; Hanes, 1937). These are known as alpha- and beta-amylase, and the enzyme capable of attacking raw starch is referred to as the "raw starch factor." Those flours which are made from ungerminated sound wheat contain beta-amylase with or without the raw starch factor. Flour made from sprouted wheat contains those enzymes present in flour made from sound wheat and in addition alpha-amylase (Sandstedt, 1938). There may be still some doubt as to the separate entity of the raw starch factor, inasmuch as it has been studied only in a mixture with alpha-amylase.

Beta-amylase, so far as is known, is present in appreciable but varying amounts, alpha-amylase is either absent or present in varying amounts, and the raw starch factor ranges in amount from little or none to appreciable quantities (Sandstedt, Blish, Mecham, and Bode, 1937; Blish, Sandstedt, and Kneen, 1938). Estimations of the amounts of the various enzymes have been carried out by modifications of the Lintner procedure (American Association of Cereal Chemists, 1935; Sandstedt, Blish, Mecham, and Bode, 1937; Blish, Sandstedt, and Kneen, 1938; Collatz and Racke, 1925; Malloch, 1929; Hills and Bailey, 1938) involving partial or complete extraction of the enzymes and evaluation of the amount by allowing either the extract or a purified preparation of it to act on a given substrate. Beta-amylase is able to convert cooked starch or starch granules which have been thoroughly disrupted in a rod mill (Stamberg and Bailey, 1939b) to maltose at a rate depending on the enzyme concentration. Beta-amylase will convert up to about 60 % of cooked starch to maltose (Hanes, 1937; Blish, Sandstedt, and Kneen, 1938). The residual dextrin material (40 %) termed erythrodextrin can be completely converted to a lower dextrin by alpha-amylase if the enzyme concentration is high enough. The raw starch factor is able presumably to convert the raw uninjured starch granules into a dextrin or maltose, the rate of conversion depending on the concentration of the enzyme (Sandstedt, Blish, Mecham, and Bode, 1937; Blish, Sandstedt, and Mecham, 1937; Blish, Sandstedt, and Kneen, 1938).

The various combinations of the amylolytic enzymes as they occur in flour offer a complex problem to the cereal chemist and one finds the interpretation of their part in the autolytic digestion of flour rendered more difficult because of the nature of the substrate on which they act.

It has been shown that the susceptibility of the starch to amylases varies in different flours (Alsberg, 1927; Mangels, 1926; Hermano and Rask, 1926; Malloch, 1929; Andrews and Bailey, 1934; Sandstedt,

Blish, Mecham, and Bode, 1937; Blish, Sandstedt, and Mecham, 1937; Eva, Geddes, and Frisell, 1937; Harris and White, 1938; Blish, Sandstedt, and Kneen, 1938), durum wheat starch being most susceptible and starch from soft-wheat flour being the least susceptible to attack. This difference may be considered to be possible of explanation in two ways. Either the raw starch granules are uniform in composition in a given sample and bear within their structure or composition some property, the alteration of which, by heredity or environmental conditions, results in a change in susceptibility of each granule of that particular starch, or the individual starch granules are not uniform but consist of a susceptible group and a resistant group. Varying amounts of each group would then give rise to differences in susceptibility for the samples.

The work done by Sandstedt, Blish, Mecham, and Bode (1937) has shown that the susceptibility of the raw uninjured starch in different samples of amylase-free flours, to an amylase containing extract of a given flour, is the same and that differences noted between samples of such inactivated flours are due to the varying proportions of the susceptible starch material which they contain.

The visible detection of starch granules which are susceptible to amylase action has been carried out by Brown and Heron (1879). More recent work by Pulkki (1938) has shown that it is possible to differentiate wheat-starch granules of two kinds by staining with iodine and congo red so that one class is coloured blue and the other partially or completely red. The latter class includes all the visibly injured granules and in addition a few other granules showing no discernible injury. Pulkki found that for a given sample of semolina and middlings material, the granules staining completely or partially red were practically all in the size class of 20μ diameter or over and that the percentage of these granules in that class is closely correlated with the fineness of the sample and with the percentage of maltose formed during autolytic digestion. He further showed that a strong malt extract acting on a sample of wheat starch resulted in a greater visible damage to each of the granules which was capable of being stained with congo red, but no increase in the total number of such granules.

I—Susceptible Starch in Australian Flours

Flours milled from Australian-grown wheats vary widely in their maltose figures (Jewell, 1935; Hickinbotham, 1936a, 1936b; Farquhar, 1938; Breakwell, 1938; Breakwell and Hutton, 1939; Bottomley, 1938), a large proportion being lower than is considered desirable. The so-called "strong" or "hard" wheats such as Pusa 4, Baringa, and

Dundee usually give a high and satisfactory maltose figure, while the "weak" or "soft" wheat varieties such as Nabawa, Free Gallipoli, and Ford give a low maltose figure.

This lack of ability to produce maltose during an autolytic digestion and its indication of the probable inadequate gassing power of the flours has been compensated for by the miller and baker through the use of sprouted-wheat supplements, and by a certain amount of alteration in milling practice.

The method of staining used by Pulkki (1938), slightly modified as noted below, seemed to offer an approach to the study of the maltose figure in relation to the susceptible starch in different samples of flour, as contrasted with that relationship in a single sample of flour ground to varying degrees of fineness.

This section of the report concerns itself with the relation of the amount of susceptible starch to variety and locality where the wheat was grown, to the maltose figure from the autolytic digestion, to severity of grinding, to protein content, and finally with the effect of injury of starch granules on the density of the granules as revealed by sedimentation experiments.

Experimental

Samples of flour made from the wheat varieties Baringa, Pusa 4, Nabawa, Free Gallipoli, and Ford, grown at three places in Victoria, and from Baringa, Pusa 4, Dundee, Nabawa, and Ford, grown at three places in New South Wales, were examined. Unfortunately not all varieties could be obtained from each place but sufficient were secured to indicate some very interesting relationships.

The Victorian flour samples were obtained from wheat supplied and milled by the Victorian Department of Agriculture. The wheat was first reduced in a Wiley mill. This stock was put through the reduction rolls of an experimental mill and sieved. The white stock was put through the reduction rolls a second time and sieved. The flour yield was 50% to 60%.

The New South Wales flour samples were obtained from the Department of Agriculture of New South Wales and were portions of experimentally milled flours prepared for use in that department.

Starch granules for staining were prepared from 10 g. of flour by washing out with water. The starch suspension was filtered through a fine silk sieve (13xxx). One-fourth of the suspension was diluted to 200 ml. One ml. of a 2% alcoholic iodine solution was added and, after 30 minutes, 5 ml. of a 1% aqueous solution of congo red was added. By the use of a projection microscope the images of the starch granules were projected on a ruled card where measurements were made with

a glass ruler at 500 magnification. Two classes of granules measuring 20μ or over in diameter were counted, those that stained partially or completely red and those staining blue. At least 3400 granules were measured for each sample.

The results are set out in Table I and indicate that there is a marked relation between the percentage of starch granules which stain with congo red and variety of wheat but no relation with the place where the wheat was grown.

TABLE I

THE PROPORTION OF STARCH GRANULES OF 20μ DIAMETER OR OVER WHICH STAIN RED WITH IODINE-CONGO RED SOLUTION

Place	Baringa	Pusa 4	Nabawa	Ford	Dundee	Free Gallipoli
	%	%	%	%	%	%
Dookie, Vic.	20.7	20.4	12.1	7.7	—	10.8
Werribee, Vic.	20.2	26.8	11.6	6.5	—	11.5
Walpeup, Vic.	23.2	20.6	10.3	7.2[1]	—	10.7
Temora, N. S. W.	25.0	17.6	8.8	7.5	15.6	—
Gilgandra, N. S. W.	26.7	19.9	6.9	5.0	12.7	—
Condobolin, N. S. W.	26.1	17.1	10.3	9.3	17.3	—

[1] Value supplied by calculation.

Autolytic digestion by the Blish and Sandstedt method (American Association of Cereal Chemists, 1935) produced amounts of maltose in the different samples which bore a marked relation to variety and a slight tendency to be higher for the Victorian samples than for the New South Wales samples. (See Table II.)

TABLE II

THE DIASTATIC ACTIVITY—AS MALTOSE PER 10 G. OF FLOUR IN 1 HOUR AT 30°C.

Place	Baringa	Pusa 4	Nabawa	Ford	Dundee	Free Gallipoli
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Dookie, Vic.	245	233	165	164	—	208
Werribee, Vic.	233	279	156	118	—	143
Walpeup, Vic.	244	219	148	96[1]	—	150
Temora, N. S. W.	258	239	118	96	203	—
Gilgandra, N. S. W.	336	235	103	66	—	—
Condobolin, N. S. W.	258	222[1]	112	84	—	—

[1] Values supplied by calculation.

For purposes of the analysis of the results, the flour samples of the four varieties Baringa, Pusa, Nabawa, and Ford grown at each of the six places were considered. The missing values (3) in Tables I and II were supplied by calculation. The total correlation, +.934, represents the overall relationship between the two factors of percentage of starch

granules which stain with congo red and the diastatic activity for all the samples in Tables I and II. By eliminating all samples of Free Gallipoli and Dundee from consideration, the overall correlation becomes +.954 (see Table III).

TABLE III

THE ANALYSIS OF VARIANCE AND CO-VARIANCE

	Degrees of freedom	Sums of squares and products			
		SX^2	SY^2	SXY	r
Total	21	1262.79	125,533	12,011.8	+0.954
Varieties	3	1136.60	107,801	11,060.3	+0.999
Places	5	7.61	3,151	30.3	+0.196
Varieties × places	13	118.58	14,581	921.2	+0.701

This is in reality a composite of each of the effects of their separate constituents. The two correlations +.999 and +.196 represent the values for the relationship between the two factors for the variety means and place means. The variety value is high and significant but it is only calculated on the basis of four means. The place value is low and not significant and therefore place of growth has little or no effect on the correlation between the percentage of granules staining red with congo red and the diastatic activity. The final correlation +.701 represents the relationship between these two factors after the variety and place effect has been removed. It is fairly high and significant, thereby indicating that apart from any variety effect there is a definite relationship between the two factors, diastatic activity and percentage of granules staining red in congo red solution.

The relationship between the percentages of granules stained with congo red and the maltose figures is set out graphically in Figure 1. The results given by Pulkki (1938) which are applicable for comparison purposes are also included in Figure 1. It is noteworthy that they show a similar relation between the maltose figures and percentages of granules stained with congo red. Those varieties such as Baringa, Pusa 4, and Dundee, requiring a high roller pressure to reduce the endosperm, have a high proportion of granules which stain with congo red and a high maltose figure. Other varieties such as Free Gallipoli, Nabawa, and Ford, which require less pressure, show few granules which stain with congo red and a low maltose figure. This constitutes evidence that it is the action of the milling processes on wheats with different inherent properties that is responsible for the variation in the maltose figure and the percentage of starch granules susceptible to staining with congo red.

As confirmation of Pulkki's work (1938), two samples of commercial

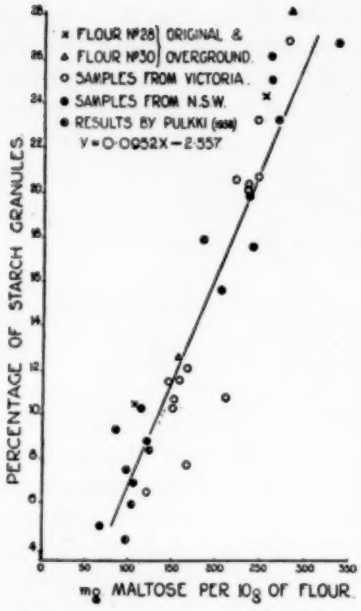
× FLOUR №28 ORIGINAL &
▲ FLOUR №30 OVERGROUND.
○ SAMPLES FROM VICTORIA.
● SAMPLES FROM N.S.W.
● RESULTS BY PULKKI (1936)
Y=0·0952X−2·557
PERCENTAGE OF STARCH GRANULES
mg. MALTOSE PER 10g OF FLOUR.

Fig. 1. The relation between the maltose formed after one hour at 30°C. and the percentage of starch granules of 20μ diameter or over which stain with congo red.

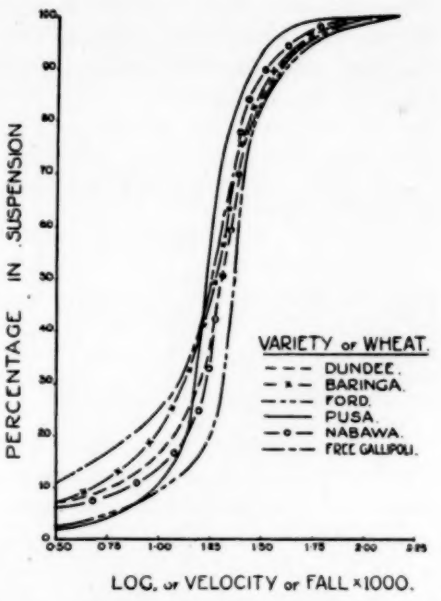

Fig. 2. Summation curves illustrating the sedimentation of starch granules from six varieties of wheat grown in Victoria, 1936.

flour were subjected to severe grinding by passing them several times through the closely set reduction rollers of an experimental mill. The data as set out in Table IV indicate the magnitude of increase both in the maltose figure and in the percentage of granules staining with congo red. The data are recorded in Figure 1.

TABLE IV

THE EFFECT OF OVERGRINDING TWO COMMERCIAL FLOURS

Sample No.	Protein on moisture-free basis	Maltose per 10 g. of flour in 1 hr. at 30°C.		Starch granules of 20μ diameter or over staining with congo red		Maltose produced in 1 hr. at 30°C. by enzyme extracted from 10 g. flour on protein-free basis	
		Original flour	Overground flour	Original flour	Overground flour	Original flour	Overground flour
	%	mg.	mg.	%	%	g.	g.
28	9.7	105	252	10.5	24.3	52.5	49.2
30	10.0	153	282	12.6	28.1	56.1	51.1

In seeking an explanation for the variation between varieties as shown in Tables I and II, one cannot neglect the possibility of there

being a difference in the size distribution of the starch granules in the flours examined. A sedimentation method was used to study the size of the starch granules. Some method of separation of the starch was necessary which would retain all the granules, both large and small. On merely washing out with water some of the small granules are left behind in the gluten. The flour was therefore treated by the method of Hartmann and Hillig (1926) in which the gluten is digested with pepsin. This was carried out in an acid solution to inhibit the amylase action during digestion.

In this separation, 25 g. of ether-extracted flour were digested overnight at 37°C. in 300 ml. of 1% hydrochloric acid containing 1.25 g. of pepsin. The starch was separated by centrifuging, the supernatant liquid discarded, and the starch resuspended in 300 ml. of the acid-pepsin mixture. After the second overnight digestion, the starch was again separated by centrifuging, the supernatant liquid poured off as completely as possible, and the starch suspended in tap water and diluted to 1200 ml. in a tall stoppered cylinder of about 6.5 cm. diameter. The suspension was maintained at 15°C. in a constant-temperature room. After thorough mixing, 20 ml. portions were taken out by pipette and placed in tared porcelain evaporating basins for drying. The depths and the times after mixing at which the samples were taken were so chosen as to cover the progress of the sedimentation to a point when 10% or less of the starch was still in suspension. Beginning with the greatest rate of fall, the first aliquot was withdrawn at 30 cm. depth after 1 minute (by stop watch) after mixing. The suspension was again mixed and the second aliquot taken after 2 minutes at 30 cm. depth. This process of mixing, allowing to stand for the appropriate time, and taking the aliquot at the proper depth was continued for the remaining samples required according to the schedule set out in Table V. The amounts of sediment in the first three aliquots agreed very closely and constituted 100% in suspension,

TABLE V

Aliquot	Time interval after mixing			Depth at which aliquot was taken	Rate of sedimentation
	hr.	*min.*	*sec.*	*cm.*	*cm./sec.*
1	0	1	0	30	0.5000
2	0	2	0	30	0.2500
3	0	4	0	30	0.1250
4	0	6	0	20	0.0555
5	0	7	28	20	0.0446
6	0	15	47	20	0.0211
7	0	22	13	20	0.0150
8	0	47	0	20	0.0070
9	2	13	20	20	0.0025

since down to this rate of fall corresponding to 0.125 cm./sec. no diminution in the concentration of starch granules had occurred at the depths and times specified. The amount of starch in each progressive sample gradually decreased until the termination of the sedimentation measurements corresponding to a rate of fall of 0.0025 cm./sec.

A slight excess of 2% ammonia (about 0.75 ml.) was added to the evaporating basins holding the 20 ml. aliquots before careful drying on a sand bath. After the main portion of the water was removed, drying to constant weight was completed by heating to 110°C. in an electric

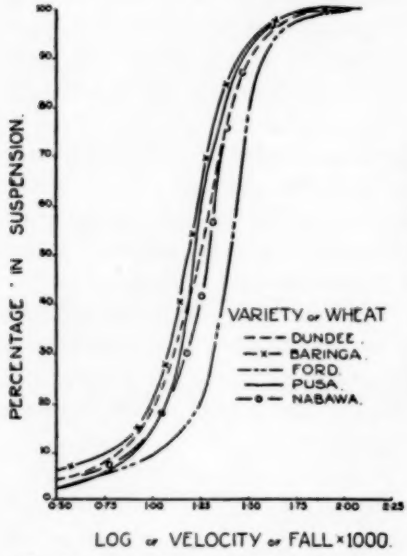

Fig. 3. Summation curves illustrating the sedimentation of starch granules from five varieties of wheat grown in New South Wales, 1936.

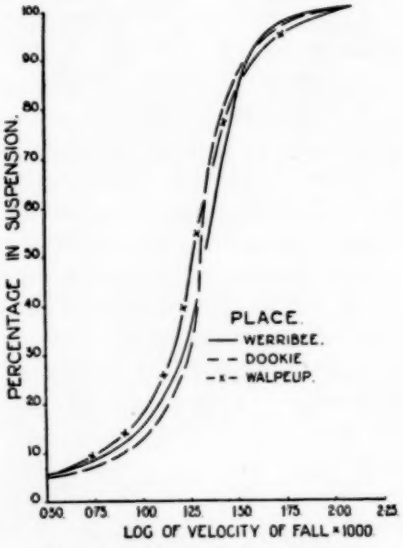

Fig. 4. Summation curves illustrating the sedimentation of starch granules from the same varieties of wheat grown at three places in Victoria, 1936.

oven. The weight of material in each aliquot was corrected for the trace of soluble material present in the sedimenting liquid by drying 20 ml. of the clear supernatant liquid obtained by centrifuging a portion of the suspension. The viscosity and density of this liquid was also determined and found to be practically constant from sample to sample.

Although the density of the starch granules in such a water suspension is not known, the rate of settling as recorded in a sedimentation curve provides a basis for comparisons of the characteristics of the starch granules obtained from different samples of flour. Sedimentation curves typical of those obtained for the starch from six different varieties of wheat grown in Victoria and five grown in New South Wales are shown in Figures 2 and 3. If the flours made from different

varieties of wheat contain different-sized distributions of granules, according to Stoke's law, such a sedimentation curve should reflect that size difference. On the other hand differences in average density of the granules from flour to flour would also be evident in sedimentation curves. The differences observed in Figures 2 and 3 between the curves is such that the time taken for sedimentation of 50% of the starch increases in the order of Ford, Nabawa, Dundee, Pusa 4, and Baringa. This, it will be noted, is in the reverse order to that observed for the extent of injury to the starch granules as shown by staining with congo red. In other words, varieties that suffer extensive damage

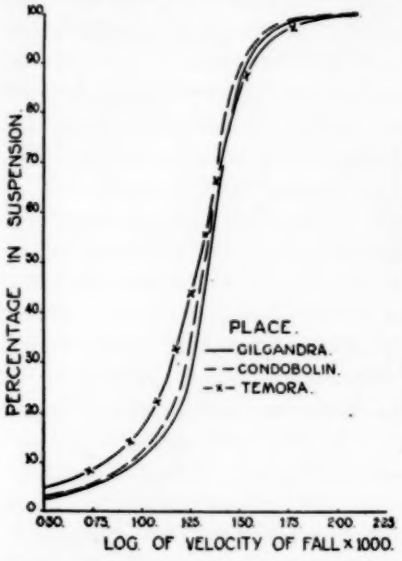

Fig. 5. Summation curves illustrating the sedimentation of starch granules from the same varieties of wheat grown at three places in New South Wales, 1936.

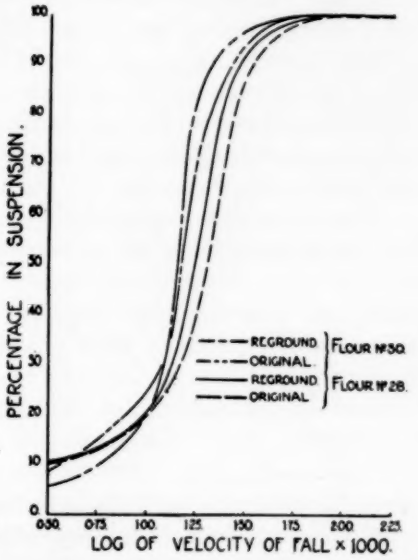

Fig. 6. Summation curves illustrating the effect of overgrinding on the sedimentation of starch granules from two samples of commercial flour.

to their starch granules during milling, settle out of a suspension more slowly than do varieties which show little damage to the granules. This is in accord with what is known regarding the swelling which a starch granule undergoes when it has been injured (Alsberg, 1938). As a result of the swelling, the diameter of the starch granule is of course increased somewhat with a consequent decrease in density.

In contrast with the marked differences noted between the sedimentation curves for the starch granules of different varieties, the influence of locality during the growth of the wheat plant is very slight (Figs. 4 and 5). This is true for both the New South Wales samples and the Victorian samples and may be due to lack of sufficient variation between the soil or climatic conditions of the six places. The examina-

tion of the same varieties from other seasons can give a partial answer to this question. In any case the similarity between the sedimentation curves in Figures 4 and 5 is paralleled by a similarity between the percent of injured granules in the flour samples from the six places.

The effect of increased injury to the starch granules was noted by examination of the sedimentation rate of the starch granules from a given flour which had been subjected to two grinding operations. The original and overground commercial flours Nos. 28 and 30, already described in Table IV, were digested with the acid-pepsin mixture as noted above and then sedimentation curves obtained (Fig. 6). The effect of increased injury to the starch granules is most noticeable when the sedimentation is only partially completed, that is, when the larger granules are being deposited whilst during the later stages of the sedimentation there is little difference between the reground flours and the original flours. The examination of the starch-granule sediment microscopically supports this in that there is no evidence of extensive amounts of starch-granule fragments in the reground flours.

The volume of the sedimented starch granules from the reground flours is greater than from the original flours. The increase in the water-binding capacity of the injured starch granules over that for uninjured granules may have an important bearing on the water-absorption figure for a flour. The moisture contents of the starch sediments from the original and overground samples of flour (No. 30) were compared after concentrating the sediment by centrifuging. All supernatant liquid was poured off and the firmly packed starch was weighed and dried at 110°C. The overground starch had a moisture content which was 8.5% higher than the original starch.

The starch from such a variety as Pusa 4 is similar to that from the overground flour No. 30, in that both contain relatively high proportions of damaged starch granules, while the starch from Ford on the other hand is similar to that from the original flour No. 30, in that both contain a relatively low proportion of damaged starch granules. In order to determine whether the similarity as noted above extends to the water-binding capacity of the starch of the different samples, these two varieties, Pusa 4 and Ford, grown at Werribee were subjected to an acid-peptic digestion. The volume of the starch from 5 g. of Pusa 4 was 9.6% greater than that from the same amount of Ford and the moisture content of the Pusa 4 starch was 12.3% higher than for the Ford starch.

Since the sedimentation method of studying the size of starch granules gave more information on the treatment to which they had been subjected in milling than it did on the actual size of the original granules, direct measurements were made. The starch granules for these

measurements were prepared by digesting 5 g. of flour with a solution of pepsin overnight, mixing the suspension well and placing small drops on a clean slide. The drop was diluted with water and spread over an area of about 2 sq. cm. and allowed to dry for three days in the air. For examination, an anhydrous liquid, *e.g.* lactic acid, was used to prevent swelling of the granules. Measurements were made at 500× using a projection microscope and a glass mm. ruler. A minimum of 500 starch granules of 8μ or over in diameter were measured in each sample. The average diameter was taken as the arithmetic mean of the long and short diameters of the starch granule.

Data were collected on the size distribution of starch granules in 16 samples of flour from 4 different varieties of wheat. A volume distribution curve was calculated on the basis of the measured diameters. Starch granules below 8μ in diameter were not counted for all the samples, so that in order that all the results be comparable, only granules of 8μ diameter or over were included in the calculation of the volume distribution for the different samples. From the data available (Dadswell and Wragge, 1937) these small granules make up about 95% of the total number of granules so that it is evident that the frequency curve for percentage of total number in relation to diameter is very much asymmetrical. However, in spite of their overwhelming numbers, the small granules constitute only 7.5% of the total volume of the starch.

Those summation volume-distribution curves in Figures 7 and 8 represent the maximum variation due to variety and place of growth for the 16 samples studied. This variation is not of the magnitude of the difference between the sedimentation curves in Figures 2 and 3.

Varietal differences among the samples examined were of about the same magnitude as the differences due to place of growth.

The protein contents of the samples as set out in Table VI bear no significant relation to the proportion of injured granules when all the samples are considered regardless of source or variety (correlation,

TABLE VI

THE PROTEIN CONTENT ON A MOISTURE-FREE BASIS

Place	Baringa	Pusa 4	Nabawa	Ford	Dundee	Free Gallipoli
	%	%	%	%	%	%
Dookie, Vic.	10.4	13.5	9.8	11.4	—	9.6
Werribee, Vic.	14.2	12.3	10.4	13.5	—	13.4
Walpeup, Vic.	12.3	13.4	12.3	—	—	15.5
Temora, N. S. W.	10.9	14.7	11.1	11.6	11.8	—
Gilgandra, N. S. W.	13.6	13.4	11.8	14.8	14.1	—
Condobolin, N. S. W.	14.5	14.9	15.0	11.6	13.0	—

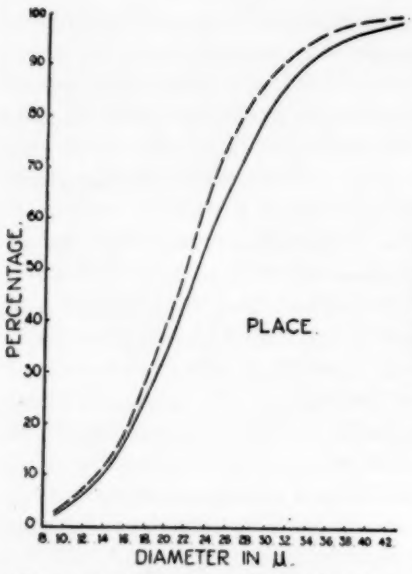

Fig. 7. Summation curves showing the maximum difference in volume distribution of starch granules in relation to diameter, as determined by direct measurements, for the same varieties of wheat grown at two places in Victoria.

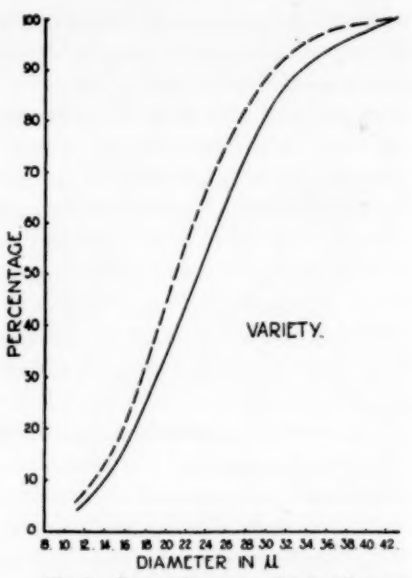

Fig. 8. Summation curves showing the maximum difference in volume distribution of starch granules in relation to diameter, as determined by direct measurements, for four different varieties of wheat grown at the same places in Victoria.

−.091). But when the samples of one given variety are considered there is a tendency for those having the highest protein content to have the fewest granules capable of staining with congo red and the lowest maltose figure. The work carried out by Breakwell and Hutton (1939) in South Australia on the relation between protein content and maltose figure indicates that whole meal from areas producing high-protein wheat has a lower maltose figure than whole meal from wheat grown in areas producing low-protein wheat. Graesser (1936) found that there was a significant negative correlation between the diastatic activity and protein content of 25 randomly chosen second-patent and straight-run Canadian Western hard-wheat flours. On the other hand work by Geddes and Aitken (1935) on flour made from 28 samples of wheat from 11 different countries indicates no correlation between diastatic activity and protein content.

It is possible that the higher protein content of some samples acts in such a way as to shield the starch granules from injury in milling, although such injury as noted in the samples reported on in this paper is more closely related to variety than to the protein content.

II—Enzyme Activity

It is believed from the evidence presented by other workers (see discussion in introduction) that beta-amylase is the most active amylase

present in normal flour milled from sound wheat, and that it is the one which is largely concerned in the formation of maltose in an autolytic digestion. Consequently a very dilute cold-water extract of the various flours studied and reported on in this paper was made and allowed to act on boiled soluble starch. It is considered that this procedure is justifiable as a measure of the enzyme concentration where the rate of maltose formation is dependent on the concentration of enzyme, as in the case of beta-amylase and the raw starch factor, *i.e.*, when little or no alpha-amylase is present.

Beta-amylase in the actual autolytic digestion of flour is considered to be acting on that portion of the starch substrate which is comparable to the starch ground in a rod mill or to boiled starch (Stamberg and Bailey, 1939b); therefore the latter was used as the substrate for assessing the enzyme activity. In order that a lack of the substrate would not be a limiting factor in the assessment of the concentration of enzymes, a large excess of the buffered boiled starch was used.

Experimental

Five grams of flour were mixed with 100 ml. of cold water and allowed to stand one-half hour with frequent shakings. One ml. of filtrate of the flour suspension was allowed to act at 30°C. on 157.5 ml. of 2% soluble starch which had been mixed with 14 ml. of water and 57.5 ml. of buffer solution which contained 12 ml. of glacial acetic acid and 16.4 g. of anhydrous sodium acetate per litre. The soluble starch was made up according to the directions given by the American Association of Cereal Chemists (1935). A blank was run on the mixture after the flour extract had been added. At the end of one hour, during which the flasks were shaken at 15-minute intervals, another sample was taken for a maltose estimation by the Blish-Sandstedt method (American Association of Cereal Chemists, 1935). The results were calculated as grams of maltose produced by the amylase in the water extract from 10 g. of flour.

An indication of the part which the activity of the enzyme may play in determining the maltose figure in the autolytic digestion of flour may be judged from observing the values for amylase strength as set out in Table VII.

The activity of the enzyme extracts varies from flour to flour and bears little consistent relation to variety except for the samples of Free Gallipoli which have a marked tendency toward the highest activity. The samples from Victoria have, in general, a higher amylase activity than do those from New South Wales, and likewise the maltose figures obtained by autolytic digestion (Table II) are slightly higher for the Victorian samples. Considering the individual samples the

TABLE VII

THE AMYLASE STRENGTH—AS MALTOSE PRODUCED BY THE ACTION OF A WATER EXTRACT FROM 10 G. OF FLOUR ON A PROTEIN-FREE BASIS, ACTING ON 2% SOLUBLE STARCH FOR 1 HOUR AT 30°C.

Place	Baringa	Pusa 4	Nabawa	Ford	Free Gallipoli
	g.	g.	g.	g.	g.
Dookie, Vic.	52.4	87.9	46.4	67.6	59.4
Werribee, Vic.	65.0	81.5	48.8	43.9	106.5
Walpeup, Vic.	68.0	80.2	59.8	—	104.4
Temora, N. S. W.	45.1	15.4	53.6	16.6	—
Gilgandra, N. S. W.	37.8	56.8	7.9	53.5	—
Condobolin, N. S. W.	25.9	—	26.9	10.4	—

over-all correlation between amylolytic activity and the maltose figure by autolytic digestion is +.206, which is not significant.

The amylolytic activity of the samples bears no relation to their protein content. Severe overgrinding reduces the amylolytic activity of the two samples of flour considered in Table IV.

Summary

Six representative varieties of wheat grown in Victoria and New South Wales have been studied.

Injury to starch granules in milling is correlated with variety but not with place of growth.

The maltose formed during the autolytic digestion of flour is positively correlated with the extent of injury to the starch granules and consequently with variety.

Two samples of commercial flour were subjected to overgrinding with a consequent increase in both the maltose figure and the proportion of injured starch granules.

An increase in the proportion of injured starch granules is accompanied by an increase in their wet volume and a higher water-binding capacity.

Measurements of the average diameter of starch granules from four different varieties grown at four places indicate that the variation in the volume distribution of the granules due to environment is of approximately the same magnitude as that due to variety.

The protein content of the flours examined bears no correlation to the proportion of injured starch granules or to the maltose figure. Within a variety however there is a slight tendency for the highest protein content to be accompanied by the lowest injury to the starch granules.

The amylolytic activity of the water extract from the flours examined varies widely and bears no significant relation to the maltose figure.

Conclusions

The results of this work indicate that different varieties of wheat require entirely different treatments by the miller in order to obtain a similar degree of availability of the starchy portion of the flour, but it is probably more feasible for them to blend the wheat of different varieties carefully in order to standardize the gassing power of flours.

A still more fruitful field would seem to be that of selection for growth of those varieties of wheat having the desired inherent properties not only as has been done in regard to protein but also on the basis of availability of the starch for enhancing the gassing power of the flour.

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COMPRESSIBILITY OF BREAD CRUMB

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Much good chemistry and physics has been expended on the physical properties of bread dough. An excellent summary of all this work has been made by C. H. Bailey (1940). Halton and Scott Blair (1937) have gone so far as to measure the viscosity and modulus of elasticity in absolute units. For dough the importance of this is evident. In fermentation, rounding, molding, proofing, and spring in the oven, dough must be capable of deformation without tearing, so that its viscosity and elasticity are among its essential qualities and its suitability for various uses depends directly upon these two qualities and upon the relation between them.

This is equally true in the larger field of biscuit, cracker, and cake doughs. In 1912 the senior author was employed as dough mixer by a large manufacturer of biscuits and crackers. Here the range extends all the way from the completely liquid suspensions of flour used for sugar-wafer batter to the hard-tack doughs that seem to resemble rhinoceros hide more closely than any other common material. The resulting baked products are very sensitive to variations in viscosity and

elasticity of the doughs. He early realized that experienced practical dough mixers perceived qualities in a dough which they did not have the ability to describe clearly in words. As early as 1913 his notes show a conscious effort to express in definite physical constants the desirable characteristics of each dough. Even at that date, before the illuminating work of Bingham on plasticity, his notes show a realization of ductility, elasticity, and stickiness as more or less distinct physical characteristics to be considered. Like so many others of that date he was groping for the ideas now so clearly expressed by Scott Blair (1939).

For the finished baked product the importance of physical measurements is not so self-evident as it is for the dough. Bread and biscuit doughs may have to flow and stretch, but the finished bread and biscuits do not have to do so to any considerable extent. Hence physical characteristics are worthy of study only to the extent that their importance can be demonstrated. Unlike the measurement of physical properties of doughs, such measurements on baked products carry the obligation to show that such measurements are worth making and have some real meaning.

Measurements of the compressibility of bread crumb seem to be justified for the following reasons:

1. Progress in general is stimulated by the development of reliable, quantitative methods. Especially in the food industries there is a general need for objective measurements to replace mere opinions with their well known shortcomings.

2. Softness in bread is recognized as a desirable characteristic. Within certain limits and other things being equal, the softer the bread the better. Therefore a method for measuring this quality conveniently is worth while.

3. Softness or compressibility is recognized as an important factor in freshness. As a result of a prolonged consumer preference study, Stateler (1936), reporting for the New York Section of the American Association of Cereal Chemists, said: "From all these tests only one factor seems to have any special significance. That factor was freshness. In bringing out that factor the experts as well as the consumer group were more nearly consistent in their conclusions." It has been shown that measurement of rate of change in compressibility is one of the best methods for following the loss of freshness or rate of staling.

4. Compressibility is shown to have diagnostic value as an indication of the approach to the optimum in several factors such as mixing and fermentation.

5. Compressibility of crumb doubtless has many points of purely theoretical scientific interest, but these are not explored in the present report.

Previous Work

Measurements of the breaking and crushing strength of biscuits and crackers were made by Davis (1921) and by Platt and Fleming (1923), and the method described was also used to measure the shortening power of fats and the softness of flours. Physical measurements on cakes were described by Platt and Kratz (1933). The softness of bread crumb was measured by Katz (1917) and his method summarized in English by Katz (1928). He used the cut surface of a loaf without removal of crust. Katz used this method to measure rate of staling. Further work along these lines was carried out by Platt (1930) who gives the details of a simple apparatus made by attaching a plunger to the bottom of one pan of a large balance. Platt used a slice of bread $2\frac{1}{2} \times 2\frac{1}{2}$ inches and $1\frac{1}{2}$ inches thick with the crust trimmed off. He describes a convenient method for carrying out this test and gives the effects of temperature, age, and varying weights on the compressibility of bread crumb. L. H. Bailey (1930, 1932) describes a simple, though not very sensitive, apparatus for the same purpose. A prism of bread crumb $1\frac{1}{2} \times 1\frac{1}{2} \times 2$ inches is subjected to a load of 500 or 1000 g. Change in compressibility measures rate of staling.

Steller and Bailey (1938) studied the effects of various factors on the staling of bread using viscosity, sedimentation, and compressibility tests. For the latter they used an apparatus similar to that of Platt. Staleness was expressed as the percentage loss of compressibility as compared with the compressibility of bread one hour out of the oven as the standard. This gave quite sensitive and concordant results. A possible objection to this method is that no data are given as actually observed. All are stated as a percentage of the findings on the bread when one hour old. Unfortunately determinations made on very fresh bread have the greatest experimental errors, due to difficulties in slicing fresh bread, difficulties in temperature control, and to the fact that at this age the condition of the bread is changing very rapidly, as can be seen from any staling curve. These errors could probably have been reduced by selecting an age of two, three, or four hours (or an average at two of these ages) to form the base from which other data would be calculated on a percentage-of-loss basis.

Two important conclusions from this paper are: (1) "Flour strength appears to be an important factor in the aging of bread crumb, the rate of staling being a function, though not linear, of the protein content of the flour." (2) "Compressibility and viscosity measurements of staleness were more consistent and uniform than the data obtained by the sedimentation method."

An interesting departure from the usual methods for measuring the

compressibility of bread was devised by Morison and Coriolis (1929). The rectangular prism of bread crumb to be tested is $2\frac{1}{2} \times 2\frac{1}{2} \times 3$ inches. This is subjected to a 7-pound (3175 g.) weight for 15 seconds and the position of the plunger noted. The weight is then removed and the bread is allowed to spring back towards its normal shape for 30 seconds. The percentage of rebound is calculated. In this case the weight is many times that used by any other investigator. The effect is made the more severe by the fact that the weight is applied suddenly, giving considerable impact before its fall is arrested. This instrument was called a "penetrometer."

The foregoing test was originally devised to determine when a loaf was sufficiently baked. It is the only objective test for this quality known to the present authors. Interesting effects on the readings with this penetrometer were also shown by the amount of fermentation, richness of formula, strength of flour, and other factors. Cathcart and Pushnik (1939) state: "A loaf that is gummy or one that is unbaked will show a low per cent of rebound. One that is well baked and seems to chew well or have good eating qualities will have a higher rebound. All these tests were performed on bread at the time of scoring. Penetrometer readings have been correlated fairly well with the score for 'Bread Quality.'" Here we have a definite statement of the connection between compressibility results and eating quality.

The object of the present paper is threefold: first, to arrive at a clearer understanding of what happens in making a compressibility test; second, to make available a number of recent improvements and refinements in methods for making compressibility measurements; third, to present the results of such measurements as affected by various factors and to point out their practical application.

Details of the Improved Method

The basic method of making the compressibility test is briefly as follows: The apparatus as depicted in Figure 1 consists of large balance having a plunger fastened to the under side of one of the pans. The piece of bread to be tested is placed on a platform immediately below the plunger with the plunger resting on the upper surface of the bread. The weight is then applied for a specified time and the amount of compression measured by means of a pointer which magnifies the movement 3.9 times. This simple instrument has been described and illustrated by Platt (1930).

Changes in details of the new method from the method described in 1930 are as follows: The face of the plunger has been enlarged from 31 mm. diameter to 36 mm., the latter having a surface of 10.2 sq. cm.

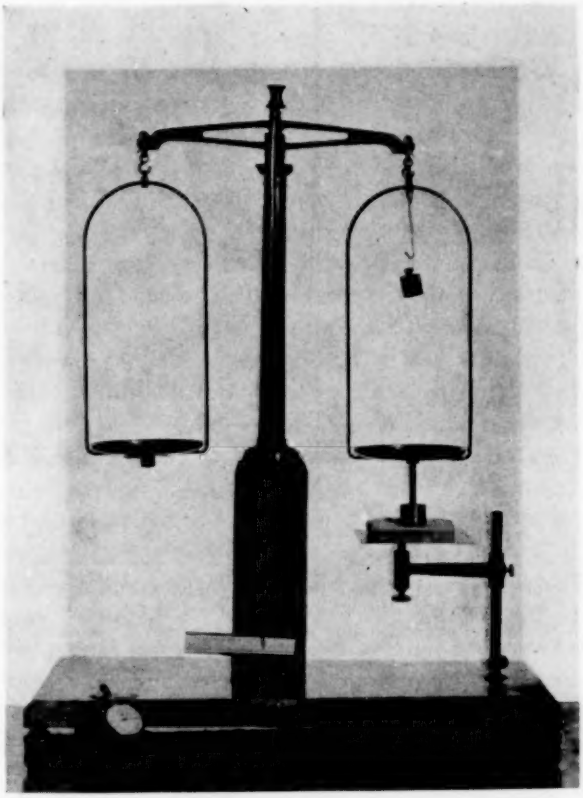

Fig. 1. Balance type of apparatus for measuring compressibility. Note plunger resting on slice of bread being tested.

The larger face is found to decrease the error because it is less affected by small local irregularities in grain and texture of the bread surface. The time of action has been reduced to 15 seconds.

The graphs of compressibility vs. time are shown in Figures 2 and 3. Evidently most of the compression takes place in the first few seconds. About 67% has occurred in the first 5 seconds and 72% in 15 seconds. After 15 seconds the pointer indicating the downward movement of the plunger on the bread is moving very slowly. The percentage error in timing and reading is negligible. There is no indication of a definite end point; neither is there any evidence that anything is gained by continuing the load for much longer periods. On the other hand there are definite advantages in a short period for each test, so that many tests can be made on bread at approximately the same age. Because of variations between slices and between loaves a rather large number of tests is necessary in order to get a broad view of the variation

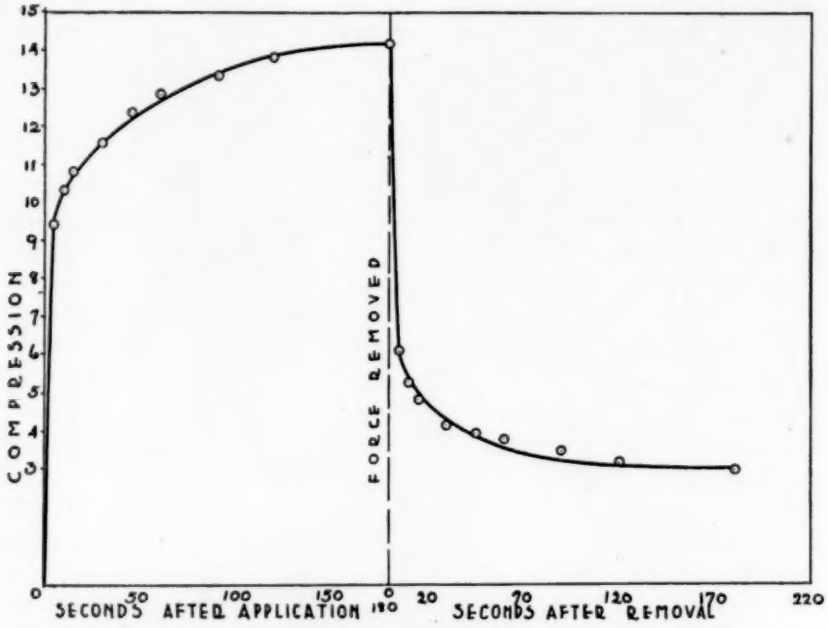

Fig. 2. Effect of time on compression after addition and removal of the force. Weight 100 g. Bread 24 hours old. Each point is the average of four slices from the same loaf.

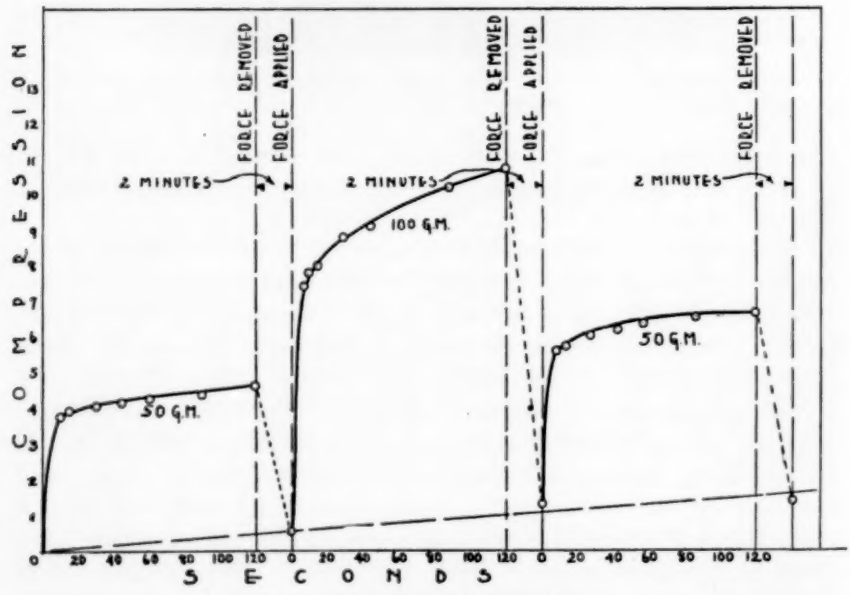

Fig. 3. Effect of repeated compression.

of compressibility in the batches under consideration and in order to increase the significance of the results.

The addition of a weight direct to the balance pan over the plunger which compresses the bread is found to introduce errors due to the jarring effect of the sudden application of the weight. One satisfactory method for avoiding this is by the use of a chain as a counterweight. The chain is removed from the left pan instead of adding a weight to the right pan (Platt, 1930). This is an entirely satisfactory method when the time of application of the stress is considerable. However, for a 15-second total application a quicker method is desirable. A quick addition of the weight without the production of a disturbing impact was accomplished by adding the weight to the righthand side of the balance by hanging it on a hook which was in turn suspended by a rubber band. The stretching of the rubber absorbs the jar, which if present causes a measurable effect on the readings.

Adjustment to zero and the final reading of the pointer are made with the aid of a large lens. The end of the pointer has been sharpened so that readings are made by estimating to tenths of a scale division. Each scale division measured by the pointer corresponds to 1/3.9 or about 0.26 mm. descent of the plunger in compressing the bread.

While compressibility tests on bread at all ages from one hour to 72 hours are of interest, the tests of most practical interest are those between 12 and 48 hours old, as this is the age at which most bread is actually eaten. Errors are also less than those made on very fresh bread.

In all the previous work on the compressibility of bread crumb already cited the tests have been made on rather thick pieces of bread usually cut as rectangular prisms with the crust trimmed off. Relatively large stresses with correspondingly large resulting strains were used. A useful improvement has consisted in making the compressibility tests on ordinary slices of commercial thickness (about ½ inch) without removal of the crust. The possibility of doing this was first pointed out by Baker (1939).

Testing ordinary slices has the following advantages over testing specially cut thick rectangular prisms of bread crumb: (1) Convenience. (2) Saving of time. (3) Great increase in the number of replicates per loaf, thereby reducing the error of the mean. (4) Considerable reduction in the error of slicing. The best commercial bread slicers cut a remarkably uniform slice with no apparent injury to the crumb from crushing or tearing. However, when cutting a piece of bread of special size and shape for testing, a considerable error may be introduced in the cutting, and special skill is necessary. (5) Bread is eaten by the slice,

so that there is real meaning in studying the slices themselves as is discussed below.

When testing laboratory loaves made in a pan of commercial size and shape, best results are obtained by slicing them for testing on a commercial slicer. However, if such is not available excellent results can be obtained by the use of a mitre box. A convenient form is shown in Figure 4. The base is made of hard wood having the same width

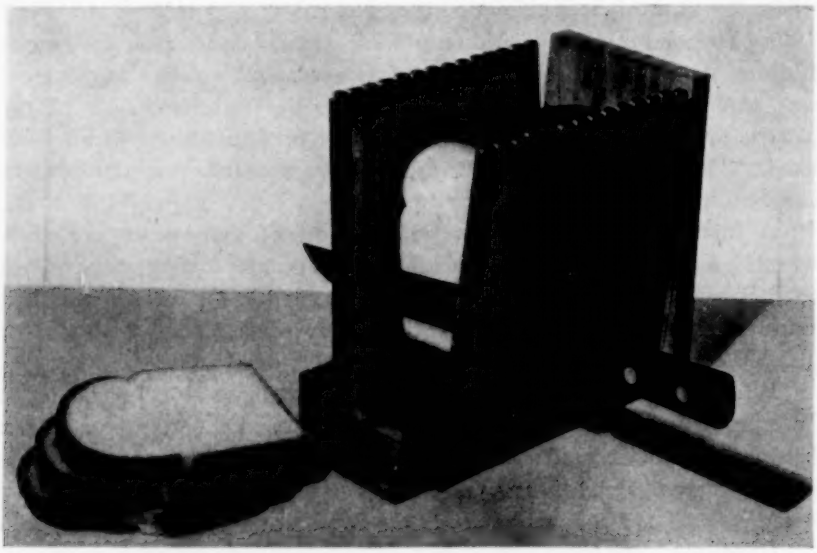

Fig. 4. Mitre box for precision slicing of laboratory loaves.

as the pan in which the bread was baked. Guides are provided by brass strips 4.5 mm. thick and 12 mm. wide. The strips are placed 1 mm. apart to allow the insertion of the bread knife. These strips are placed at the same angle from the vertical as the sides of the bread pan in which the loaf was baked. A Burns bread knife with saw-tooth edges does the best job in cutting the bread. Such a mitre box exactly fits the loaf and supports it while it is being cut. The box produces slices with parallel surfaces and of uniform thickness.

Bread purchased in the grocery store which has been commercially sliced and wrapped often contains slices which are somewhat warped so that they do not lie flat. Many are "cupped" so that their two surfaces are concave and convex respectively. If such a slice is placed under the plunger with the concave side downward and the weight is then applied, results much too high will be obtained. This is because the plunger must first travel some distance in flattening out the slice

and in pushing the lower surface of the slice firmly down against the supporting platform before actual compression of the bread occurs. This error may be avoided by turning the slice so that it is concave upward, in which case the lower surface under the plunger may be caused to lie flat against the supporting platform.

Another method which is even more satisfactory is to place a metal disk about 1.5 mm. thick and 38 mm. in diameter on the platform supporting the slice of bread directly under the plunger. Such a disk supports the lower surface of the slice so as to prevent its bending downward under the plunger when cupped. In all slices whether cupped or not this disk tends to localize the effect of the stress directly under the plunger and lessens the spreading of the effect of this stress to remoter parts of the slice.

One result of measuring compressibility on slices of bread of the usual size was to bring out the differences in the compressibilities of different parts of the same slice—a point first brought to the writer's attention by J. C. Baker. These variations are of three kinds: first, random differences due to lumps, holes, and other accidental small local variations in texture—most of them quite evident to the eye or finger; second, the expected loss in compressibility due to a too close approach to the crust; and third, more interesting than these, the consistent difference found in most normal slices of bread. Twist bread introduces additional irregularities not considered here.

It might be expected that the geometrical center or the point farthest removed from the crust would be the softest, but such is seldom the case. In pan bread the softest part is usually in the center about one third of the way from the bottom of the slice. In 27 slices taken from 3 loaves, all about 20 hours old, measurements were made in succession on the upper third, the center, and the lowest third of the slice. Results at two different ages are shown in Figure 5. At 20 hours the lowest third is found to be 1.9 times as compressible as the upper third. At 50 hours the factor was 1.3 in this case. This distinct difference in the compressibility of different parts of the same slice makes it important that when differences in compressibility are being considered, the same place on each slice should be measured in every case.

When a slice is under the plunger for test the area of the slice which is being compressed can be marked by touching the surface of the bread at the edge of the plunger nearest the observer with an inked pen. This can be done while the test is being made without affecting the results. The small ink spot makes it possible to apply the plunger to the same location if a second test is required.

In previous studies of rate of staling it has always been necessary to study one loaf or group of loaves to establish data for the fresh bread,

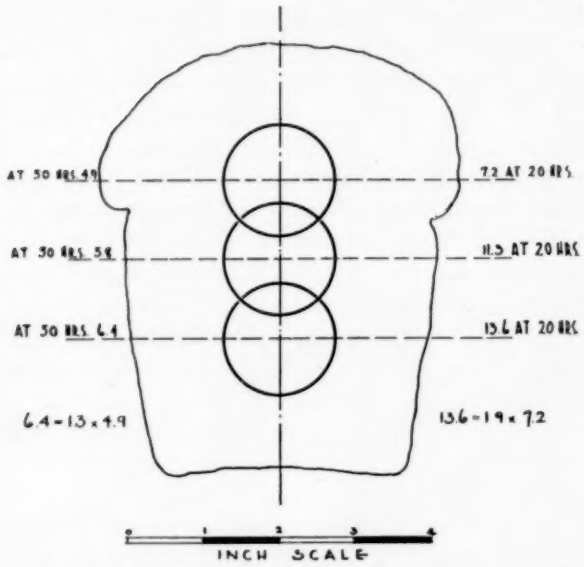

Fig. 5. The lowest third of a slice is usually the softest. Figures represent the average compression of all slices from three loaves, each slice tested at the three points shown.

and then a different loaf or set of loaves to establish the data for the bread at each succeeding age. This method really measured the compressibility of one group of loaves when fresh and of another group out of the same batch when stale. Thus the accidental differences between loaves were included in the experimental error, decreasing the significance of the difference between fresh and stale readings accordingly.

By testing slices, and preserving these same slices without physical damage in their original order and without loss of moisture, it is possible to test the same loaves and the same slices and the same spot in each slice at two or more ages. Such a method evidently eliminates many errors and improves the reliability of the results.

A convenient way to store slices for these tests is in a bread pan of the same size and shape as the one in which the loaf was baked. The pans are stored in tin boxes with a tight (but not completely air tight) cover. All storage and tests are carried out in an air-conditioned bakery where a temperature of 78° and a relative humidity approximately 40% are maintained. The bread is out of the boxes only long enough to make a test and the surface of each slice is exposed only long enough to make the test on that slice and the next. As the testing of the slices proceeds the slices are repiled in the same order. Loaves are of course numbered and any individual slice can be relocated if desired because its numerical order has been preserved.

Contrary to expectations the slices in the center of the loaf are not found to be softer than those nearer the ends. Even when the two slices nearest each end are excluded, the slices toward the end tend to be the most irregular in compressibility and are usually softer (Tables I and II). Cupping, if present, is also more apt to be found in com-

TABLE I

VARIATIONS FROM SLICE TO SLICE IN SAME LOAF, BETWEEN DUPLICATE LOAVES, AND BETWEEN FORMULAS

Compression			
Water		4% powdered skimmilk	
Loaf A	Loaf B	Loaf P	Loaf Q
11.2	13.0	15.5	14.9
9.7	11.8	11.9	14.0
9.5	9.3	11.9	11.0
8.1	7.9	10.3	12.1
8.0	8.2	13.6	11.0
8.9	9.9	13.0	13.0
9.8	10.6	14.9	14.4
10.0	12.1	17.0	17.2
12.0	13.7	11.9	16.1
AVERAGE EACH LOAF			
9.7	10.7	13.3	13.7
AVERAGE OF DUPLICATES			
10.2		13.5	

TABLE II

REPRODUCIBILITY OF RESULTS OF RETESTING SAME SLICES IN THE SAME PLACE

Compression		
1st test	2nd test	Difference
9.0	9.0	0.0
13.0	13.0	0.0
12.1	12.0	0.1
10.5	10.9	0.4
11.1	11.0	0.1
11.9	11.8	0.1
10.5	10.0	0.5
9.2	9.0	0.2
9.8	9.7	0.1
8.4	8.5	0.1
8.3	8.0	0.3
7.9	7.2	0.7
9.0	9.0	0.0
11.0	10.7	0.3
AVERAGE		
10.1	9.99	.2

Average difference between tests is 0.2/10 = 2% of mean.

mercially sliced and wrapped loaves in the slices near the end. For this reason we have excluded the two slices nearest each end in short loaves, and the three slices nearest each end in long loaves. One possible reason for the softer slices near the ends may lie in molding conditions. In general the dough near the ends receives less crowding and is less tightly molded than that at the center. The principal reason for recognition of these variations in compressibility is to permit the avoidance of errors which arise when these discrepancies are not taken into consideration.

If we make a chemical test on two samples of a solution taken from the same bottle and find widely varying results, we rightly conclude that our testing method is unsatisfactory. However, if we find wide variations in the compressibility measurements of different slices from the same loaf, we cannot at first be sure whether this indicates a real variation in the slices, or a considerable error in our method, or both. The best plan found for checking the error of the method and thus for selecting the method having the least error, is to test every slice in one or more loaves and then to retest immediately the same slices at the same marked spots. The average of the differences between the tests on the same slice is an indication of the precision of the method.

When large forces are allowed to act for relatively long times and when the weights are applied without care in the elimination of impact the crumb of the bread may be permanently changed by the first test so that the second will show an increase in average compressibility. However, when the test is carried out as described above there is practically no destruction of crumb structure. The second test is found to average 1.05 times the first one. This correction can be made if desired, but is usually negligible. The variation to be expected under favorable conditions between duplicate tests on the same slices is shown in Table II. The variations to be expected from slice to slice, from loaf to loaf, and from batch to batch when the batches are made with different formulas are shown in Table I.

The J. C. Baker Compressibility Apparatus

An entirely different form of apparatus for measuring compressibility has been designed by J. C. Baker (1939). The apparatus is shown in Figure 6. The slice of bread, placed as shown, is acted on by a plunger *A*. Adjustment is made for the thickness of the slice by means of the nut *B*. Scale *C* reads slice thickness direct in millimeters. When the plunger is depressed, the amount of the depression is multiplied 10 times and is read direct in millimeters on scale *D*. The depression is caused by the rotation of a small drum *E*, which is actuated

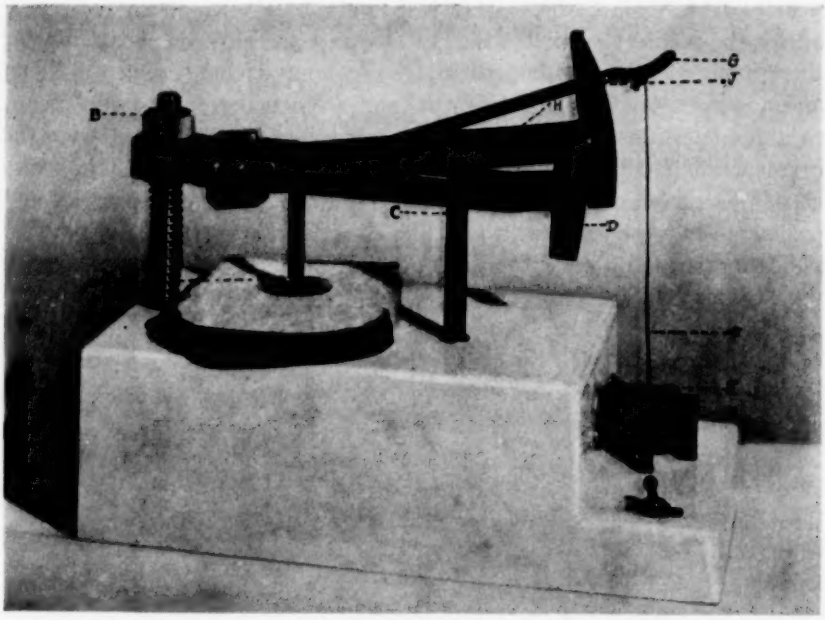

Fig. 6. Apparatus of J. C. Baker for the measuring of compressibility of bread. Stress and strain are indicated simultaneously on scales *J* and *D* respectively.

slowly and uniformly by a motor and shaft located below the bed of the apparatus. The string *F*, which is wound on the drum, serves to depress lever *G*. This lever is connected to lever *H* through a coil spring (not shown because located behind scale *J*).

When the apparatus is in use the amount of the stress at any moment is given on scale *J* and the amount of the corresponding strain or depression of the plunger at the same moment is indicated on scale *D*. The drum *E* is made so that it will slip on the shaft. By this means the drum may be adjusted, wound, unwound, or stopped with the fingers, regardless of the motion of the shaft. By turning the drum manually in this way, either stress or strain may be held constant over a period of time and the resulting change in the other variable noted. The plunger face is removable so that various sizes and shapes can be used. A diameter of 36 mm. is convenient.

It is helpful to place a disk 38 mm. in diameter and 1.5 mm. thick on the bed of the apparatus, directly under the plunger so as to support the underside of the slice firmly in spite of warping or shrinkage.

Determination.—Mark the bed of the apparatus so that each slice may be placed in such a position that the plunger will act in the same relative place on each. Loosen the string. By means of the nut *B* and by pushing down on the counterweight raise the plunger. Insert

a slice of bread in the proper marked location. By means of the nut *B* adjust the pointers on both scales to zero in a uniform manner. Throw the electric switch, thereby starting the motor, which revolves the drum and depresses lever *G* at a uniform rate. When this lever is depressed to a predetermined point on scale *J* (that is, to a certain stress) the corresponding depression (or strain) is read on scale *D*. The stress chosen as the standard stopping point on scale *J* for any series of experiments is the largest which can be used without causing the pointer indicating strain on scale *D* to run off the scale. This point varies with the softness of the bread being tested. When the proper point has been reached the reading is taken on scale *D*. The further movement of the drum is arrested by grasping it with the fingers, and the switch is turned off. The drum is unwound to relieve the tension and to allow the levers to return to their normal positions.

This apparatus like the first is intended for the measurement of all slices in several loaves. The precision is approximately the same as that of the first apparatus. For this type of determination no stop watch is necessary.

This apparatus can be used for certain other types of measurement. For example concurrent readings of stress and strain may be made and curves drawn. Also the force on the plunger may be raised to a certain point, and this force maintained constant (as shown by the pointer on scale *J*) over a measured number of seconds by manual adjustment of the drum. Changes in the resulting depression or strain (as shown by the pointer on scale *D*) are plotted against time. Or the strain may be held constant and the resulting diminution in stress measured against time.

What Happens when Bread Crumb is Compressed

The general characteristics of bread crumb under compression have been described in the references already given. At this time a little closer consideration of the mechanism is justified. We should be reminded that in testing the compressibility of bread crumb we are not testing one homogeneous material under one stress such as a mild steel bar under tension, for example. The structure of the bread evidently plays an important part. Considering this elaborate and delicate structure it is indeed surprising that bread recovers such a large percentage of its original form after ordinary compression. This shows that little permanent damage from tearing or breaking has been caused by the compression.

It is evident that in a compression test of a slice of bread only a part of the crumb subjected to stress is under compression. Some is subjected to bending stresses. Some parts of the slice, for example

the upper surface near the plunger, is actually under tension. The changes in the shapes of the cells under the action of the plunger are shown in Figures 7 and 8. In this case some slight deviation from normal conditions is caused by cutting away part of the slice in order to show the action directly under the center of the plunger.

In his book on "Elasticity, Plasticity and Structure of Matter" Houwink (1937) reproduces two diagrams from Burgers (1935). These show two strain-time diagrams resulting from the application and

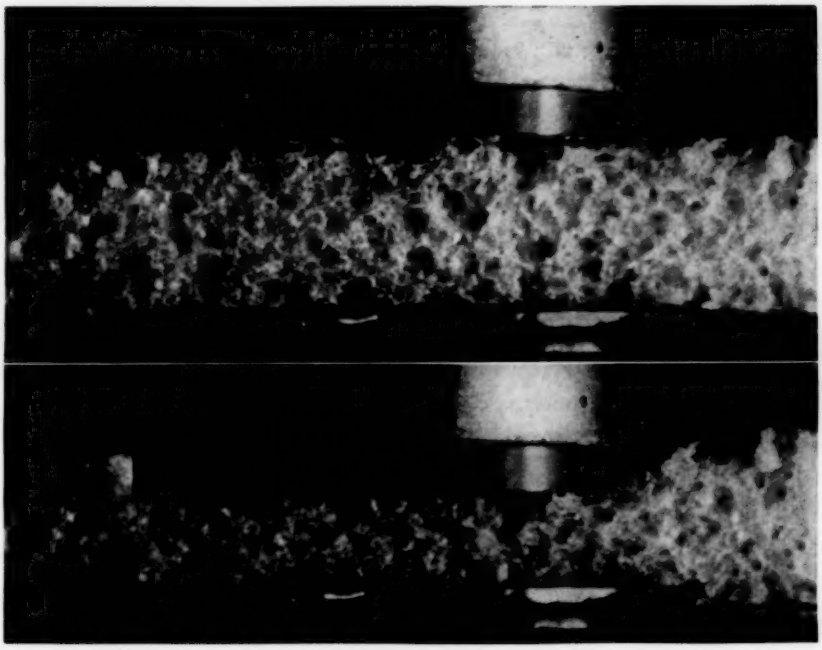

Fig. 7 (*Above*). Section of slice under plunger before compression.
Fig. 8 (*Below*). Section of slice under plunger during compression. Same bread as shown in Figure 7.

subsequent removal of a stress to a system having both elastic and viscous properties. In the first instance the elastic and the plastic elements are connected in series. In the second instance they are connected in parallel. It is interesting and suggestive to note that the shape of the curve where the elastic and the viscous elements are connected in parallel closely approximates curves for bread crumb both for compression and for recovery, as for example in our Figure 2. It is evident that in the crumb both properties are present and that they can be measured separately as has been done by Halton and Scott Blair for dough. In the crumb the elastic properties greatly predominate.

By cutting a rectangular prism of bread of known size from a slice,

the compressibility of the separate piece can be determined without the influences of the other parts of the slice which are not under the plunger and the modulus of compressibility determined in absolute units. Results depend upon the conditions under which the test is made. Testing bread 48 hours old and allowing the force to act 1 minute, a modulus of 6.5×10^4 was obtained. This compares with a modulus for dough obtained under different conditions and reported by Halton and Scott Blair (1937) of the order of 4.0×10^4. It must be remembered, however, that the dough was tested as an essentially continuous material, while the bread was tested with the open structure which is characteristic of bread crumb. A continuous mass of bread crumb material would of course be quite different in all physical characteristics.

Effect of Several Variables on Compressibility

Compressibility measurements in the past have been primarily concerned with measurements of rate of staling. The effect of various factors other than staling will now be considered. All of the data here shown were derived from laboratory (but not pup) loaves baked in a pan of commercial size and shape. The standard formula and method are given below. This was varied as indicated for each factor studied. In all cases the amount of water was varied where necessary to give doughs of the same consistency.

FORMULA

Sponge	Dough	Total, %
215 g. flour	135 g.	100
142 g. water	89 g. (variable)	66±
— powdered skim milk	21	6.0
— Sugar	17.5	5.0
— Salt	7.0	2.0
— Lard	14	4.0
5.25 g. yeast	—	1.5
1.75 g. yeast food	—	0.5

Method

Sponge is mixed in batches taking 640 g. of flour at a time in a McDuffee-type dough mixer at low speed for 4 minutes. Temperature 85° F. Ferment 3 hours. Dough is mixed in McDuffee-type mixer at low speed 4 minutes. Stands 15 minutes, and is then mixed in Fleischmann mixer 3 minutes at 120 rpm. Dough is scaled at 18 oz., is put through the molder twice at 15-minute intervals, panned and proofed at 90° F. to constant height—usually 55 to 60 minutes, and baked in rotary oven for 35 minutes at 425° F.

The effect of varying the amount of skim-milk solids with appropriate variations in the added water is shown in Figure 9. Within the limits shown, increasing the skim-milk solids gives very significant increase in the softness of the crumb.

The effect of differences in the baking quality of the powdered skimmilk used is shown in Figure 10. This difference in the softness of the resulting bread still persists even when mixing methods were changed

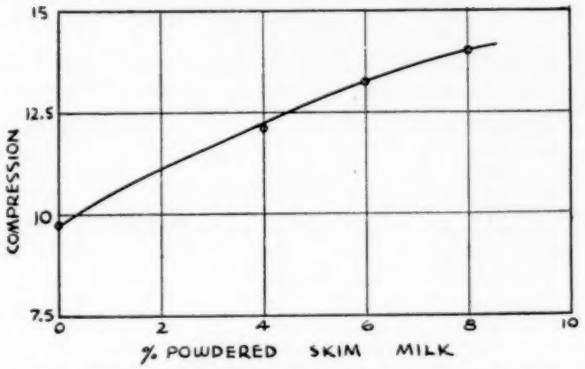

Fig. 9. Effect of increasing the percentage of powdered skimmilk.

in a search for a method which would give better results with the poorer milk. Measurements of the softness of the bread could, in fact, be used as a method for measuring the comparative baking quality of two samples of the same bakery raw material.

In our opinion the fairest and most significant procedure for com-

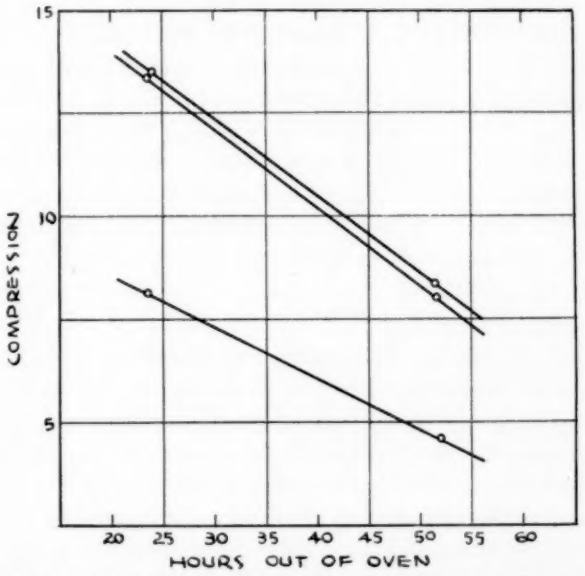

Fig. 10. Effect of powdered skimmilk having good or poor baking quality. Each line represents bread made from a different lot of powdered milk. Each point is the average of all slices from three loaves.

paring baking quality of two doughs is to take conditions of absorption, mixing, and fermentation which are optimum for each, thus comparing each at its optimum. This is certainly the objective striven for in commercial baking.

TABLE III

EFFECT OF VARIOUS FACTORS ON COMPRESSIBILITY OF BREAD

Fermentation		Time of bake	
Straight doughs	Compression	Time	Compression
Young (45 min.)	8.1	Short (25 min.)	16.3
Correct (2 hrs., 45 min.)	13.1	Medium (35 min.)	9.8
Old (4 hrs., 45 min.)	10.1	Long (45 min.)	8.6
Sugar		**Shortening**	
Amount	Compression	Amount	Compression
Low, 2%	13.0	Low (2% lard)	10.5
High, 6%	13.9	High (6% lard)	12.4

As is shown in Table III, increasing shortening causes a very significant increase in compressibility. An increase in sugar from 2% to 6% causes an increase in compressibility which is barely significant, if at all, when measured 20 hours out of the oven.

Compressibility is of course greatly affected by the degree of bake. Rather extreme conditions are shown in the same table. In this case the bread baked 25 minutes would have been considered barely salable. In the bread baked 45 minutes the crust was well browned, but not burned. When varying the amount of bake, it is *not* true of course that the softer the bread the better. Reference has already been made (Morison and Coriolis, 1929) to a method for measuring the degree of bake based upon the recovery of the crumb of bread after severe compression. The present apparatus could evidently also be used for setting up a standard as to when bread is sufficiently baked, and for measuring the adherence to such a standard.

To study properly the relation between fermentation and compressibility would require a comprehensive survey of different degrees of fermentation accompanied by various combinations of related conditions such as mixing, etc. No such extensive survey has yet been attempted. It seems probable, however, that a measurement of the compressibility of the crumb could be used as a guide to assist in approaching the optimum in fermentation, other conditions such as ingredients, degree of bake, etc., remaining constant. The effect of rather extreme conditions is shown in Table III. Maximum compressibility under conditions otherwise suitable seems to be associated with optimum fermentation.

The remarks just made regarding fermentation apply also to mixing. In the case of mixing, however, we have had somewhat more experience. Some of the results are shown in Figure 11. In this case compressibility

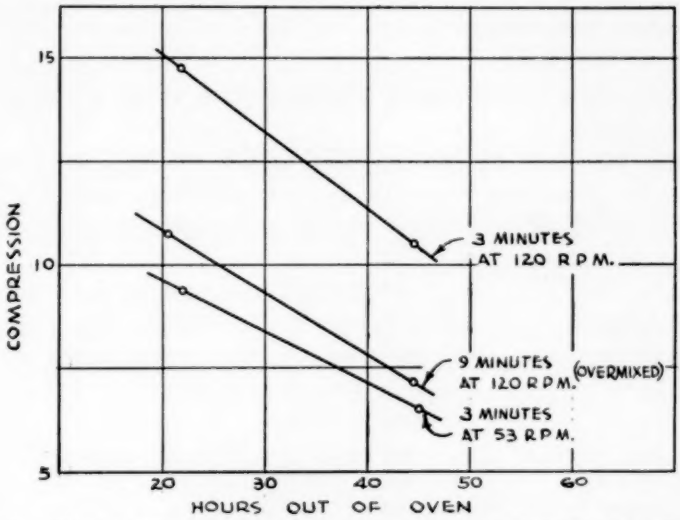

Fig. 11. Effect of mixing. Remix of sponge doughs given four minutes in a Hobart mixer at slow speed, plus added mixing in a Fleischmann mixer as shown. Each line represents bread from dough mixed as indicated. The superiority of the bread represented by the uppermost line is noticeable.

measurements first indicated that there was room for improvement in the texture of our laboratory loaves. This fact led directly to changes in mixing methods. Measurements of the compressibility of bread from dough mixed in different ways guided us to the optimum mixing now used. Further experiments in mixing gave no additional improvement in softness, affording a rational basis for the belief that the revised laboratory mixing method was near the optimum. It is suggested that measurements of compressibility while keeping all other factors constant, afford one of the best criteria for judging optimum mixing time.

Conclusions

With proper refinements the compressibility test for bread crumb is capable of considerable precision. Some important refinements are described and a method for estimating the precision is given.

A new apparatus for carrying out this test is described, which gives simultaneous readings for stress and strain.

By the revised methods, compressibility tests can be carried out on commercially sliced bread. This test is therefore available as a commercial control. It can be used effectively to detect variations in texture from slice to slice and from loaf to loaf and to indicate the existence of lumps.

The following ingredients or procedures increase the softness of bread crumb, other factors being held constant:

Optimum mixing	High skim-milk solids.
	High baking quality of milk used.
Optimum fermentation	High sugar (to a very small extent).
Reduced amount baking	High shortening.

The baking quality of the skim-milk solids used has a marked effect on compressibility.

Other factors being held constant, compressibility can be used as measure of closeness of approach to optimum dough mixing. Unsuspected opportunities to improve dough-mixing conditions can often be detected by this test.

Rate of change in compressibility of bread has long been recognized as one of the best means for measuring rate of staling. By the improvements here suggested the significance of such measurements is greatly increased.

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MODIFICATION OF THE BAILEY–JOHNSON METHOD FOR MEASUREMENT OF GAS PRODUCTION IN FERMENTING DOUGH[1]

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(Read at the Annual Meeting, May 1940)

The measurement of gas production in fermenting dough has in recent years become a common practice in many cereal laboratories. Numerous and varied devices and procedures for such measurements have from time to time been described, such as those of Bailey and Johnson (1924), Kent-Jones (1939), James and Huber (1928), Fornet (1930), Jørgensen (1931), Markley and Bailey (1932), Blish, Sandstedt, and Astleford (1932), Elion (1933), Brabender (1934), Sandstedt and Blish (1934), Landis (1934), Eva, Geddes, and Frisell (1937), Malloch (1939) and Bailey (1939). Of these methods, the procedures of Sandstedt and Blish (1934) and Bailey and Johnson (1924) appear to have been most widely used.

Since 1932 apparatus for carrying out multiple determinations of gas production in fermenting doughs has been in use in several General Mills, Inc., laboratories. This equipment, similar in principle to that of Bailey and Johnson (1924), comprises a constant-temperature water bath and from 6 to 18 individual gasometers, depending upon the requirements of the particular laboratory. A detailed report of this apparatus, which has for many years been called a "Fermeter," has not previously been published, but it seems unnecessary to describe it completely in this paper since it is essentially similar to apparatus previously

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reported by Bailey and Johnson (1924), Eva, Geddes, and Frisell (1937), and Bailey (1939).

The "Fermeter" and the apparatus described by Bailey (1939) differ only in certain minor particulars. We have preferred to use glass Mason jars as fermentation vessels, replacing the ordinary zinc top with one of spun brass of the same shape. A tubulature in the center of the lid is connected with heavy-wall rubber tubing leading to the gas-collecting burette. A cast lead washer (approximately 500 grams) is placed on the lid to keep the fermentation vessel immersed in the constant-temperature water bath.

The CO_2 liberated during dough fermentation is collected in a 100-ml. burette with inverse graduation and a two-way stopcock. When the test is started the stopcock is set to collect the CO_2 in the burette. By reversing the stopcock when the burette is filled, the gas can be discharged and the burette reset at zero, while keeping the fermentation vessel closed.

The above description will serve to show the fundamental principle of the Fermenter with reference only to certain details in which it differs from the apparatus described by Bailey.

The prime purpose of this paper, however, is not to describe the equipment but rather to describe a modification of the procedure for measuring gas production in fermenting doughs. Two years ago in this laboratory we modified the original Bailey and Johnson technique in several respects, developing a method of increased simplicity and convenience without loss in precision.

When the Fermeter was first used for measuring gas production, a normal 100-g. flour dough without sugar was mixed, fermented, and punched in the usual manner until three hours' fermentation time had elapsed. An aliquot of the dough representing 40 g. of flour was then placed in the Fermeter and gas production measured over a period of two hours. The value obtained with flours of satisfactory gassing power was of the order of 200 to 250 ml.

In carrying out the modified determination, 14 g. of flour is placed in the bottom of a half-pint Mason jar, preferably heaped on one side; 10 ml. of yeast suspension containing 4 g. of yeast per 100 ml. is pipetted into the jar and a smooth dough mixed with the aid of a small spatula. The lid is screwed firmly on the jar, which is placed in the constant-temperature water bath. The time of mixing is regarded as zero fermentation time.

The stopcock of the gas burette may be closed and gas collected from the beginning of fermentation or it may be left open during the initial two hours' fermentation to permit escape of carbon dioxide to the out-

side atmosphere. At the choice of the operator, determination of total gas production or production during any interval can be made.

It has been found that for most normal flours the rate of gas production during the first two hours of fermentation is essentially constant, and accordingly a satisfactory differentiation of gassing rate of various flours may be obtained by recording gas production during the last three hours of a five-hour fermentation period. A series of typical data is shown in Figure 1.

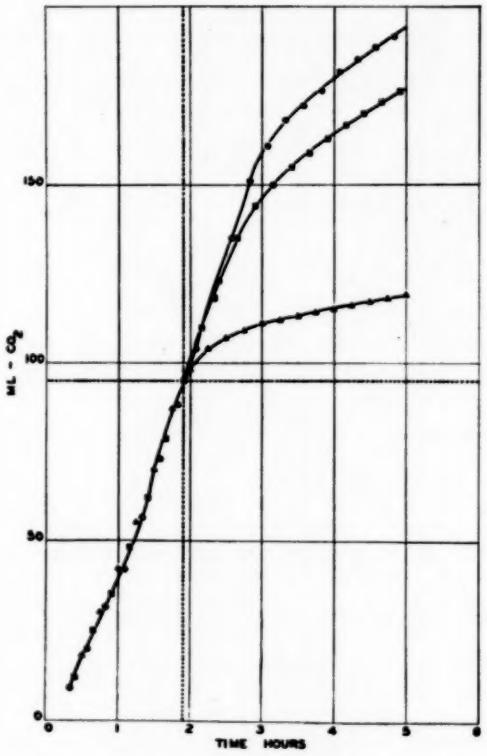

Fig. 1. Typical gas-production curves.

Since it has been found that small changes in pressure do not affect the rate of fermentation in doughs, the water level inside and outside the burette need be equalized only when readings are made, or when the capacity of the burette is reached and it must be discharged and reset.

For convenience in making the determination and recording values which can later be compared, a standard procedure has been adopted in our laboratories. The test is made with 14 g. of flour mixed into a

dough as described above. The dough is fermented in the constant-temperature bath at 30°C. After a lapse of two hours, during which gas is discharged to the atmosphere, the stopcock is closed and the gas produced is measured during the succeeding three hours. The gas production, measured in milliliters during this three-hour period, is recorded as the " Fermeter value " of the flour. Experience with a wide variety of flours, both natural and supplemented with malt flour, has shown that Fermeter values can be expected to range from 40 to 140 with a standard error of $\pm$ 2.5.

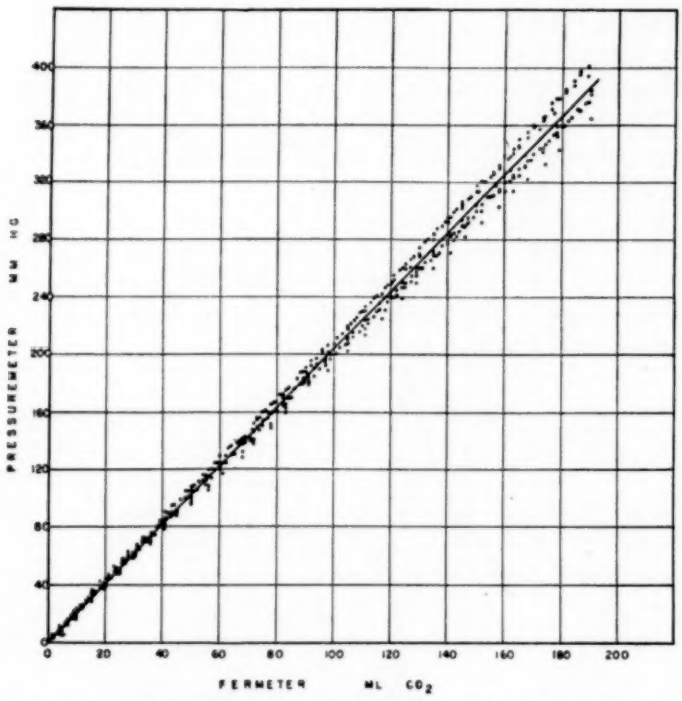

Fig. 2. Comparison of values secured with the Fermeter and with the pressuremeter.

It has been interesting to compare the " Fermeter value " with the pressuremeter values obtained by the Sandstedt and Blish procedure (1934). It has been shown by Eva, Geddes, and Frisell (1937) that measurements of gas production by the Bailey-Johnson and pressuremeter techniques are highly correlated. In order to ascertain the relationship between the gas-production values obtained in the pressuremeter and in the Fermeter, dough aliquots were scaled to provide the equivalent of 10 g. of flour for the pressuremeter and 14 g. of flour for the Fermeter.

Figure 2 shows the results of such comparative measurements. The individual points shown in this graph were obtained with 16 doughs, readings being taken on the Fermeter and on the pressuremeter at different time intervals. It will be seen that the correlation is extremely high. Calculation from the data above gives $r = +.998$. Results for the one method may be translated into terms of the other from the following regression equation calculated from these data: mm. mercury $= 0.75 + 2.03$ (ml. CO_2). Knowing the gas production value for a definite fermentation time interval for either the pressuremeter or Fermeter, one may calculate the corresponding value for the other method by use of the equation given above. The corresponding standard error of prediction, 6.9 mm. mercury, indicates that such translation of values may be done with satisfactory accuracy.

Summary

A rapid volumetric method for determining rate of gas production in fermenting dough is described. When applied to flour, gassing power is reported as "Fermeter value," expressed in terms of CO_2 evolved during the last three hours of a five-hour fermentation period.

Values obtained in the Fermeter are highly correlated with similar values obtained with the pressuremeter.

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RELATIONS BETWEEN WHEAT MALT DOSAGE, FLOUR DIASTATIC ACTIVITY, AND GASSING POWER [1]

F. C. HILDEBRAND [2] and W. F. GEDDES [3]

(Read at the Annual Meeting, May 1940)

In recent years the use of malt supplements by the milling and baking industries has created a need for suitable methods of determining the activity of such materials. Leatherock, McGhee, and Giertz (1937) suggested that the relative activities of malted wheat flours could be estimated "by adding various percentages of the different malt flours to an untreated flour, and determining the maltose before and after treatment." Davis and Tremain (1938) elaborated upon this technique by comparing malt supplements in terms of the quantities required to give a definite level of response. These authors showed that such estimations of "malt value" could be made by adding varying increments of the material to be tested to a suitable untreated base flour, determining the diastatic activity or gas production, and estimating from graphs of the data thus secured the amount required to give an arbitrarily selected level of either measure. This technique was found to be necessary since a curvilinear relation exists between malt dosage and diastatic activity or gas production. Sandstedt (1938), employing the same malts at the dosages calculated by Davis and Tremain, obtained reasonably uniform gas-production values with a different base flour.

In their studies, Davis and Tremain found a high correlation (+ .96) between "malt values" determined from diastatic activity and gas-production data but they noted that with some malts, and above a certain activity range with all treated flours, the evaluations based on diastatic activity were higher than would be predicted from the results of gas production measurements. However, for practical purposes, and

[1] Paper No. 18, Journal Series, General Mills, Inc., Research Laboratories; No. 1816, Scientific Journal Series, Minnesota Agricultural Experiment Station.
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at the levels to which flours are generally treated, these discrepancies were considered to be of no great significance.

In the course of an investigation on the effects of wheat type, protein content, and malting conditions, respectively, on the properties of malted wheat flour (Geddes, Hildebrand, and Anderson, 1940), certain modifications were made in the technique proposed by Davis and Tremain which appear to the authors to afford greater simplicity and convenience. The data thus secured also provided the opportunity for a critical examination of the relative utility of diastatic-activity and gassing-power measurements as bases for the estimation of malt activity.

Experimental

For the major studies, the experimental material comprised a series of 140 blends of 32 malted wheat flours with a Southwestern patent flour of low diastatic activity (166 mg. maltose per 10 g. flour). The malted wheat flours were experimentally produced during the course of an investigation by Geddes, Hildebrand, and Anderson (1940) and represented a wide range in amylase activity.

In addition, a second and a third series were prepared. In the second series, the lower-activity malts from the group referred to above were composited and varying amounts of the composite added to the base flour previously mentioned. In the third series, varying increments of a commercial malted wheat flour were added to a different Southwestern patent flour, also of low diastatic activity (130 units).

Gas-production determinations were carried out in duplicate in an apparatus similar to that described by Sherwood, Hildebrand, and McClellan (1940) using 14 g. flour and 10 ml. yeast suspension containing 0.42 g. fresh compressed yeast. The total gas production for zero to five hours was recorded. Diastatic activity was determined in duplicate by the ferricyanide procedure as modified by Sandstedt (1937). All weighings of malts and blends were made on a 13.5% moisture basis.

In the first series, each of the malted wheat flours was added to the common base flour at levels of 0. 25%, 0.50%, 0.75%, and 1.00% and diastatic-activity and gassing-power determinations were carried out on the resulting blends. In the instance of 12 of the malts which were found to be of low activity, additional determinations were made on blends containing 2.5% malt flour. In the second series, the composite of the low-activity experimental malts was added to the base flour previously mentioned in 0.2% increments from 0.2% to 3.2%. In the third series, the commercial malted wheat flour was added to a different Southwestern base flour at several dosage levels varying between 0.025% and 1.75%.

Results

The relation between malt-flour dosage and gas production was found to be consistently nonlinear, an observation which is in accord with that of Davis and Tremain (1938). Examination of the data, however, revealed that gas production was proportional to the logarithm of the dosage. Typical examples of these relationships are shown in Figure 1, which also includes the data for a larger number of dosages described above as the second and third series.

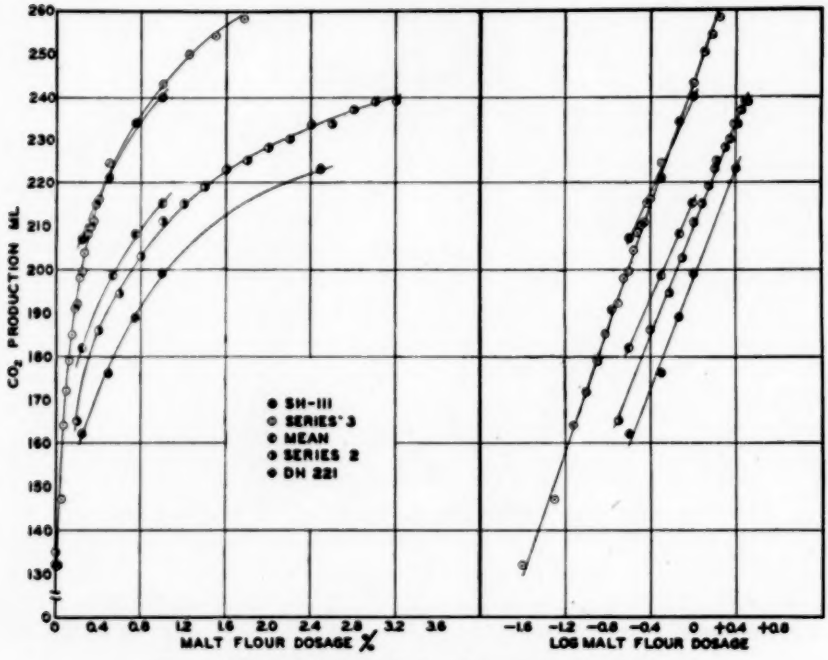

Fig. 1. Relation between malt flour dosage and gas production.

Diastatic activity also was found to bear a curvilinear relationship to dosage, as shown in Figure 2. In this instance, however, the relation was not a logarithmic one but was best expressed by a quadratic equation of the general form $D = a + bx - cx^2$ where D is the diastatic activity and x the dosage.

In order to provide as accurate a basis as possible for estimating the dosage of each malt required to produce a given level of diastatic activity or gassing power, regression equations were calculated for the duplicate sets of determinations for each malt. In computing these equations from the gassing-power data, the labor involved was materially reduced by expressing the dosages as logarithmic functions and calculating the

appropriate linear regressions. Unfortunately, this procedure, as has already been shown, could not be used with the diastatic-activity data and accordingly it was necessary to fit individual quadratic equations.

As a basis for estimating malt activity, a level of 280 mg. of maltose was selected for calculations based on the diastatic-activity data. In

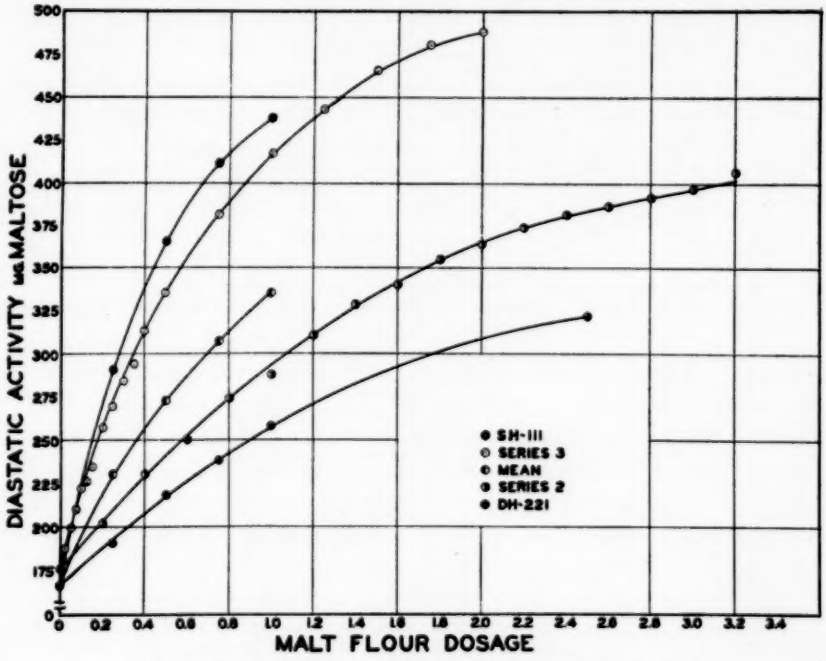

Fig. 2. Relation between malt flour dosage and diastatic activity.

order to establish an equivalent level of gassing power, the regression equation for gassing power on diastatic activity—again a curvilinear relationship—was calculated for the major series of 140 blends. From the equation thus computed, it was found that with the particular base flour employed and under the conditions of our determinations, a level of 204.7 ml. CO_2 was equivalent to 280 mg. maltose.

The amount of each malt required to give the above levels of diastatic activity and gas production was computed from the respective individual regression equations for these two measures on malt-flour dosage. In this manner, duplicate estimates of the malt dosages required to give the selected levels of both diastatic activity and gas production were secured. The means of these duplicate determinations are recorded in Table I. Casual inspection of these values indicates fairly satisfactory agreement between the dosages estimated from the two types of meas-

TABLE I

MALT FLOUR DOSAGE TO GIVE 280 MG. MALTOSE OR 204.7 ML. CO_2— MEANS OF DUPLICATE DETERMINATIONS

Treatment[1]	Hard red spring				Amber durum			
	High protein		Low protein		High protein		Low protein	
	DA[2]	GP[2]	DA	GP	DA	GP	DA	GP
	%	%	%	%	%	%	%	%
111	0.21	0.23	0.28	0.30	0.27	0.32	0.24	0.30
112	0.20	0.25	0.24	0.30	0.28	0.31	0.27	0.29
211	0.29	0.35	0.30	0.31	0.49	0.46	0.45	0.45
212	0.30	0.30	0.29	0.28	0.44	0.49	0.40	0.40
121	0.86	0.88	1.53	1.53	0.74	0.77	0.86	0.93
122	1.27	1.17	1.52	1.54	0.89	0.87	1.10	1.04
221	1.29	1.40	1.37	1.35	1.30	1.30	1.64	1.39
222	1.56	1.55	1.51	1.59	1.41	1.31	1.29	1.38

[1] Malting treatment—cf. Geddes, Hildebrand, and Anderson (1940).
[2] DA = dosage calculated from diastatic activity data; GP = dosage calculated from gas production data.

urement. The data were analyzed statistically and certain of the pertinent constants are given in Table II. The high correlation (+ .992) between the paired values for each malt shows that the two types of measurement give closely parallel results; moreover the difference between the mean values for the two methods over all malts of 0.006% is not statistically significant. The relative precision of the two methods

TABLE II

COMPARISON OF DIASTATIC ACTIVITY AND GAS PRODUCTION MEASUREMENTS AS BASES FOR MALT FLOUR EVALUATION

	Dosage calculated from	
	DA data	GP data
	%	%
Mean	0.786	0.792
Mean difference between duplicates	0.0421	0.0429
Standard error of single determination	0.0527	0.0469
Correlation between dosages by each method: $r = +.992$ (1% pt = 0.449)		

for estimating malt dosage is given by a comparison of the respective standard errors; these were tested and found not significantly different. Taking the average standard error as approximately 0.05%, the precision of estimate for both methods is roughly 0.1%. In other words if the means of duplicate determinations for two malts do not differ by this amount, they would be regarded as being of equal activity. This accuracy can hardly be regarded as satisfactory in the instance of active

malts which need be added only in small amounts. However, the precision with which malt dosage can be estimated increases with increasing malt activity. Thus for the active malts, 111 to 212 inclusive, the standard error for both methods of estimation is 0.017% as compared with 0.068% for the less active malts 121 to 222 inclusive. Thus for malts requiring dosages of from 0.20% to 0.45% to produce a flour of 280 units of diastatic activity, the precision is increased to within 0.034%.

In order to test the reliability of this general technique for estimating malt dosage, the percentages of each malted wheat flour computed from the gas-production data shown in Table I were added to the base flour previously employed. Gas-production determinations were carried out in duplicate on the resulting blends, and the mean values for each blend are recorded in Table III. The mean gas production over all blends is

TABLE III

MEAN GAS-PRODUCTION VALUES FOR FLOUR BLENDS DIASTATED WITH EQUIVALENT QUANTITIES OF MALTED WHEAT FLOURS

Treatment	Hard red spring		Amber durum	
	High protein	Low protein	High protein	Low protein
	ml.	*ml.*	*ml.*	*ml.*
111	205	207	207	208
112	207	206	208	205
211	205	206	207	204
212	205	203	205	204
121	207	206	205	206
122	206	205	203	205
221	206	206	204	206
222	206	207	203	206
	Mean = 205.5 ml.			

205.5 ml., which agrees closely with the calculated level of 204.7 ml. of CO_2. Moreover, the variation between blends was less than that between duplicates (standard error of a single determination 1.8 ml.). It may therefore be concluded that the technique employed provides a highly satisfactory means of estimating malt dosage and also therefore of measuring relative malt activities.

While it has been shown that either gas-production or diastatic-activity data may be used interchangeably in estimating malt dosage under the conditions described above, such alternate use is contingent upon the selection of equivalent levels of these measures. In this connection it is of interest to examine the relationship between diastatic activity and gas production found in this study. The paired values for the 140 blends of the first series are shown in Figure 3.

While the correlation based on the assumption of a linear relationship is quite high ($r = +.954$; 1% pt. $= 0.213$ approximately) it is clearly evident that the regression is curvilinear. After several trials in fitting various types of curves, it was found that the data were best represented

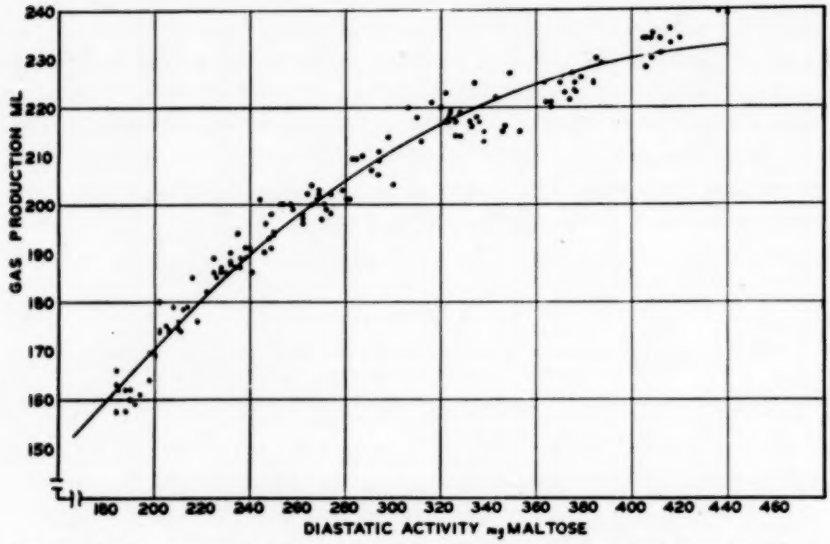

Fig. 3. Relation between diastatic activity and gas production for flour blends made by adding increments of malted wheat flours to a common base flour.

by a quadratic equation of the general form $G = a + bD - cD^2$ where G is gas production, D is diastatic activity, and a, b, and c are constants. The curve thus fitted is that given in Figure 3. When allowance was made in this manner for nonlinearity the correlation coefficient between these variables was increased to + .979.

Discussion

The data presented show that if suitable care is taken to select equivalent levels of diastatic activity and gas production, malt dosage may be estimated from either measure interchangeably and with equal precision, although the use of diastatic-activity data is much less convenient. In the case of gas-production values, linear regressions may be calculated after expressing malt dosage as a logarithmic function, whereas with diastatic-activity measurements, calculations of quadratic equations are necessary—a procedure which is much more laborious.

In the routine application of this technique, it is altogether unlikely that one would wish to resort to the calculation of individual regression equations. Indeed, where gas-production measurements have been employed it would appear that one could dispense with such calculations

without material loss in accuracy. If dosage levels are expressed as logarithms, and are plotted against the corresponding gas productions, a straight line may be drawn through the points with reasonable accuracy; if necessary the required dosage may be determined by extrapolation.

In working from a curvilinear relation, a number of determinations must be carried out in order to establish accurately the form of the curve, or a smaller number must be made at levels of activity falling close to and on both sides of the arbitrarily selected standard activity level. Since such procedures necessarily involve a relatively large number of analyses, the use of logarithmic plotting suggested above seems to promise increased convenience and simplicity to attain equivalent accuracy.

In recent years several papers have appeared in the literature dealing with the relation between diastatic activity and flour gassing power and it is becoming increasingly evident that an exact parallelism does not exist between these variables—formerly regarded by many workers as synonymous for practical purposes. Thus it has been shown that the relation between these measures is influenced by variations in substrate, such as for example in sucrose content (Blish, Sandstedt, and Astleford, 1932), and in starch susceptibility (Karacsonyi and Bailey, 1930). Other workers (Landis and Frey, 1936; Bottomley, 1938; Fisher, Halton and Hines, 1938), have suggested that the different conditions under which the two tests are carried out is an important factor affecting the correlation between these measures. In the light of these views, the direct comparison of gas production and diastatic values shown in Figure 3 is of particular interest since the measurements were made upon essentially the same substrate. Actual determinations of sucrose and reducing sugars made on a number of the flour blends selected to represent extremes of malt activity and dosage, showed that there was no measurable variation in sugar content. Accordingly it must be concluded that factors in addition to variations in substrate operate to cause a variable relation between diastatic activity and gassing power. Even with an extremely uniform substrate, the increase in gas production per unit increase in diastatic activity becomes progressively less with increasing activity. This lends further emphasis to the views advanced by several workers, as for example Landis and Frey (1936) and Eva, Geddes and Frisell (1937), that these two measures cannot be regarded as synonymous.

Summary

Thirty-two experimentally produced malted wheat flours of widely varying amylase activity were evaluated by a technique similar to that suggested by Davis and Tremain (1938). The malt flours were blended

at varying levels with a common base flour of low diastatic activity, and diastatic-activity and gassing-power determinations were carried out on the resulting blends.

Gas production for any given malt was found to vary directly as the logarithm of the dosage, while the relationship between dosage and diastatic activity was best expressed by a quadratic equation.

The amount of each malt required to produce 280 mg. maltose and the equivalent level of five-hour gas production (204.7 ml. CO_2) was calculated from the individual regression equations. The dosages thus calculated from diastatic-activity and gassing-power measurements were not significantly different and the precisions of the two techniques were equal. The precision of estimate increases with increasing malt activity; for malts requiring dosages of from 0.20% to 0.45% the precision is within 0.34%.

Flour blends prepared with dosages estimated from gassing-power data gave a mean production of 205.5 ml. of CO_2, the difference in the values for the individual malt flours being less than the error in the determination of gas production.

Estimation of malt activity is most conveniently and simply made from gas-production data by plotting ml. CO_2 against the logarithm of dosage. The quantities of malt required to produce the selected level of gassing power may be read from the straight line thus obtained.

The relation between diastatic activity and gassing power was curvilinear, although the substrate was essentially the same throughout. This observation supports the view that the different conditions under which the two tests are carried out is an important factor, aside from variations in sucrose content and starch susceptibility, affecting the relation between these measures.

Acknowledgments

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COMPARISON OF METHODS FOR THE DETERMINATION OF PROTEOLYTIC ACTIVITY. II. STUDIES ON MALTED WHEAT FLOURS[1]

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(Read at the Annual Meeting, May 1940)

Early studies of methods of estimating proteolytic activity in cereal products have been briefly reviewed by Hildebrand (1939). In an investigation reported at that time, the author compared rate-of-gelation, viscometric, and formol titration procedures. It was concluded that the two methods first mentioned gave results appreciably different from techniques based on the measurement of proteolysis end products by formol titration, presumably because dipeptidase and/or polypeptidase activity had an appreciable effect on the results obtained by the formol titration methods, whereas with the rate-of-gelation and viscometric procedures proteinase activity alone was estimated. As between the two latter methods, the rate-of-gelation technique seemed preferable since it gave better differentiation between samples, although it is not well adapted to general use because of its requirement of relatively elaborate and costly apparatus.

[1] Paper No. 16, Journal Series, General Mills, Inc., Research Laboratories. Subcommittee report, 1939–40, Committee on Methods of Analysis.

Recently Ayre and Anderson (1939) have modified a technique suggested by Northrup (1932) and have applied the method in its modified form to the determination of proteolytic activity in barley malts. This method is based on autolytic digestion of the material to be tested, precipitation of the protein with trichloracetic acid after one and three hours' digestion and determination of the unprecipitated nonprotein nitrogen by a Kjeldahl procedure. The increase in nonprotein nitrogen is taken as a measure of proteolytic activity.

The experiment here reported was planned to afford a comparison between the Ayre and Anderson procedure and the rate-of-gelation method of Landis and Fry (1938), using malted wheat flours as the material investigated.

Experimental

Twelve samples of experimentally produced malted wheat flours, selected so as to give a reasonably wide range of proteolytic activity, were used in this study. These samples were portions of the material investigated by Geddes, Hildebrand, and Anderson (1940). A complete description of the origin of these samples is given in the paper cited.

TABLE I

PROTEOLYTIC ACTIVITY OF MALTED WHEAT FLOURS

Sample	Landis and Frey method—milliunits per g.	Ayre and Anderson method—mg. nonprotein N per 100 g.
ML 211	0.81	64
	0.69	68
DH 222	1.09	95
	1.03	102
ML 121	1.07	70
	1.31	74
MH 212	1.29	111
	1.40	106
DL 211	1.66	143
	1.65	133
MH 122	1.71	111
	1.63	119
DH 211	1.65	131
	1.75	129
ML 112	1.80	169
	1.98	167
DH 122	1.97	155
	1.96	147
DL 112	2.07	201
	2.14	211
DL 111	2.16	191
	2.20	163
DH 111	2.98	251
	2.95	241

The Ayre and Anderson procedure as outlined in their original presentation was used without modification. The Landis and Frey (1938) rate-of-gelation technique was employed embodying the modifications suggested by Hildebrand (1939).

The results obtained are shown in Table I. In order to facilitate comparison, the results by both methods were reduced to the same numerical basis. The ratio between the mean values for all samples was computed and the data for the Ayre and Anderson method were multiplied by the factor thus obtained. The data recalculated in this fashion

TABLE II

PROTEOLYTIC ACTIVITY OF MALTED WHEAT FLOURS

(Values reduced to same numerical basis)

Sample	Landis and Frey method—milliunits per g.	Ayre and Anderson method—mg. nonprotein N per 1.22 g.
ML 211	0.81	0.78
	0.69	0.83
DH 222	1.09	1.16
	1.03	1.25
ML 121	1.07	0.86
	1.31	0.90
MH 212	1.29	1.36
	1.40	1.29
DL 211	1.66	1.75
	1.65	1.62
MH 122	1.71	1.36
	1.63	1.45
DH 211	1.65	1.60
	1.75	1.58
ML 112	1.80	2.06
	1.98	2.04
DH 122	1.97	1.89
	1.96	1.80
DL 112	2.07	2.46
	2.14	2.58
DL 111	2.16	2.33
	2.20	1.99
DH 111	2.98	3.07
	2.95	2.94

are given in Table II. Table III shows estimations of the error and differentiation between samples given by the two methods.

Discussion

While it is evident that the number of samples tested is insufficient to permit drawing of more than tentative conclusions, nevertheless the data serve for a preliminary estimation of the correlation between the two procedures and should be of value in guiding future studies.

TABLE III

	Landis and Frey method	Ayre and Anderson method
Mean, all samples	1.706	1.706
Range	0.69–2.98	0.78–3.07
Variation between samples, mean square	7.6033	9.3770
Variation between duplicates, mean square	0.0720	0.0989
F	115.1	103.4
Correlation between results by both methods	$r = +.956$	

The high degree of correlation between results indicates that both procedures measure the same type of activity. It has been assumed (Landis and Frey, 1938; Hildebrand, 1940) that the rate-of-gelation technique affords an estimate of proteinase activity which is substantially unaffected by the presence of other proteolytic enzymes. On the basis of the results given in this study, the same assumption may be made for the Ayre and Anderson procedure.

It may be seen from the values given in Table III that the experimental error of the two procedures is essentially the same. The difference between duplicate errors is too small to be significant with the limited amount of data employed. Similarly, it may be seen from the F values that both methods have approximately the same power to differentiate between samples. Since the performance of both methods is essentially the same, the Ayre and Anderson technique seems preferable because of its comparative simplicity and convenience.

Summary

The Landis and Frey rate-of-gelation and Ayre and Anderson precipitation methods for proteolytic activity have been compared, using twelve experimentally produced samples of malted wheat flour.

Both methods give essentially similar results and have approximately the same experimental error and ability to differentiate between samples.

The Ayre and Anderson technique is to be preferred because of its greater simplicity and convenience.

Acknowledgment

The author is indebted to Philip M. Sautier for assistance in the studies with the Ayre and Anderson method.

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COLLABORATIVE STUDY OF A RAPID METHOD FOR THE DETERMINATION OF FLOUR PIGMENTS[1]

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(Read at the Annual Meeting, May 1940)

The determination of carotenoid pigments in flour has generally been conducted by extraction with a mixture of naphtha and absolute ethyl alcohol (93 parts to 7 parts) according to the methods of Ferrari (1933), or Geddes *et al.* (1934), as outlined in Cereal Laboratory Methods (1935). According to these procedures, overnight standing (16 to 18 hours) is required to obtain complete extraction, and the method is thus not suited to purposes of mill control. A rapid procedure involving high speed stirring has been described by Coleman and Christie (1926) but does not appear to have met with general favor. Recently, Binnington, Sibbitt, and Geddes (1938) suggested the use of water-saturated n-butyl alcohol as a solvent, and Binnington and Geddes (1939) describe a rapid method for flour, involving the use of this solvent in conjunction with a 15-minute extraction period. This method yields results of a higher order than the naphtha + alcohol procedure, thus necessitating a mental adjustment on the part of the operator to a higher scale of values. The rapidity and convenience of the procedure, however, are such as to suggest its wider employment, and the Committee on Methods of Analysis therefore undertook a collaborative study of this method.

Nine collaborators participated, and three samples of bleached and three of unbleached flour were circulated. Detailed directions for preparation of the extracts were supplied; in the instance of the new method these followed the authors' procedure and in the case of the naphtha + alcohol technique, which was employed as a control, the

[1] Subcommittee report, 1939–40 Committee on Methods of Analysis. Paper No. 21, Journal Series, General Mills, Inc., Research Laboratories.

official A. A. C. C. procedure was followed. Specific directions for preparation of the extracts *only* were detailed, and each collaborator was requested to employ whatever method was available for estimating the pigment content of the extracts so obtained. Whenever possible, it was requested that more than one method be employed, and it was further suggested that a visual comparison against potassium chromate standards, using mercury vapor illumination, be used if facilities permitted. In addition, it was requested that duplicate or triplicate determinations be carried out on each sample.

The pigment content of flour, whether bleached or unbleached, is subject to reduction upon storage, and as all collaborators could not be expected to analyze the samples at the same time, steps were taken to secure an estimate of such losses in the instance of the flours employed in this study. For this purpose, the samples were stored at room temperature in cans with tight-fitting lids, tests being conducted from time to time. With water-saturated butanol, the drop in pigment content did not exceed 0.10 ppm. at the end of 30 days' storage, and with naphtha + alcohol, a maximum of 0.12 ppm. was found in 44 days. With the exception of one collaborator, all tests were conducted within 28 days and these results may therefore be considered to be substantially free from error due to alteration of the sample. One collaborator was unable to analyze the samples until a 61-day period had elapsed, and since his results suggested that some loss of pigment had occurred, they have been omitted from the statistical comparisons. Several other groups of results were also eliminated upon the collaborators' own statement that the accuracy was doubtful due to difficulties in calibrating and standardizing the instruments employed. For these reasons, the summarized results and statistical analyses presented are based upon the means for two collaborators in the instance of each method of estimation.

Because of the fact that various methods of estimating the pigment content of the extracts were employed by the different collaborators, it is possible to examine the results not only from the standpoint of solvents and extraction procedures, but also with reference to methods of estimation as well.

In Table I, the mean results for each flour are presented, together with certain statistical comparisons.

The spectrophotometric values for which means are shown in Table I were secured with a König-Martens spectrophotometer equipped with a mercury-vapor light source and a Bausch and Lomb Martens-type photometer, mercury-vapor light source, and Corning filter isolating the 4358 A.U. line of the mercury spectrum. Both sets of readings

TABLE I

COMPARISON OF SOLVENTS AND OF METHODS OF ESTIMATING PIGMENT CONTENT OF EXTRACTS—MEAN VALUES

	Water-saturated butanol				Naphtha + alcohol (93 : 7)			
Flour	Spectrophotometer	Visual	Photoelectric	Mean	Spectrophotometer	Visual	Photoelectric	Mean
A	0.84	0.77	0.86	0.82	0.62	0.55	0.52	0.56
B	1.61	1.45	1.61	1.56	1.14	1.04	1.17	1.09
C	1.10	0.95	1.00	1.02	0.74	0.65	0.70	0.68
D	2.94	2.78	3.03	2.92	2.31	2.11	2.36	2.23
E	3.38	3.30	3.53	3.40	2.71	2.52	2.76	2.62
F	3.45	3.27	3.62	3.45	2.77	2.58	2.86	2.69
Mean	2.22	2.09	2.28	—	1.72	1.58	1.73	—

Correlation between results by water-saturated butanol and naphtha + alcohol extractions: $r = +.9925$ (1% pt. = 0.212 approx.)

Regression equations:
Carotene ppm. (butanol) = 0.21 + 1.219 carotene ppm. (naphtha + alcohol).
Carotene ppm. (naphtha + alcohol) = 0.808 carotene ppm. (butanol) −0.15.

Standard error of prediction:
Carotene (butanol) from carotene (naphtha + alcohol) = ±0.14 ppm.
Carotene (naphtha + alcohol) from carotene (butanol) = ±0.11 ppm.

were made at 4358 A.U., the transmittancies obtained being computed to carotene employing the values 1.6632 and 1.91565 for the specific transmissive indices of carotene in water-saturated butanol and naphtha + alcohol respectively.

Visual results were obtained by matching against potassium chromate standards, employing unfiltered mercury arc or vapor radiation as an illuminant, and using a standard series procedure for matching according to the technique outlined in Cereal Laboratory Methods (1935).

Both sets of photoelectric results were secured with Evelyn photoelectric colorimeters equipped with glass filters having a peak transmission at 440 millimicrons and a transmission range of approximately 60 millimicrons. These instruments were standardized against a spectrophotometer using flour extracts, the same calibration curve being employed for both.

In addition to the results summarized in Table I, two collaborators reported values secured with KWSZ photoelectric photometers. These results differ widely from the mean values tabulated above and will be considered separately.

The results obtained in this study may be conveniently considered under two headings: (1) comparison between solvents, and (2) comparison between methods of estimation.

Comparing solvents, it will be noted that the rapid method yields results of a definitely higher order of magnitude and thus direct comparisons are not possible. The extremely high correlation between the two procedures indicates that both techniques give relatively similar results. In order to ascertain whether the relation between values given by the two solvents is affected by variation in method of estimating pigment content or by differences between laboratories, regression coefficients were calculated for individual sets of data. In no case was there a significant difference.

It is of further interest to compare the general equation relating values obtained by the two solvents studied with that given by Binnington, Sibbitt, and Geddes (1938). In the present study the equation derived is:

Carotene ppm. (butanol) = 0.21 + 1.219 carotene ppm. (naphtha + alcohol)

while the latter authors, working with a series of 150 hard red spring wheat flours obtained the expression:

Carotene ppm. (butanol) = 0.14 + 1.2377 carotene ppm. (naphtha + alcohol)

Table II shows a series of data selected at random, which affords a comparison between these two equations.

TABLE II

COMPARISON OF OBSERVED VALUES WITH VALUES COMPUTED FROM TWO REGRESSION EQUATIONS [1]

	Butanol		
Naphtha + Alcohol	Equation No. 1	Equation No. 2	Observed
	Carotene p.p.m.		
0.50	0.82	0.76	0.85
0.60	0.95	0.88	0.78
0.63	0.98	0.92	0.91
0.70	1.06	1.01	1.05
0.74	1.11	1.06	1.06
0.74	1.11	1.06	1.13
1.13	1.59	1.54	1.56
1.14	1.60	1.55	1.66
1.15	1.61	1.56	1.68
2.25	2.95	2.92	3.04
2.36	3.09	3.06	2.84
2.38	3.11	3.09	3.17
2.66	3.45	3.43	3.41
2.77	3.59	3.57	3.35
2.80	3.62	3.61	3.70

[1] Equation No. 1 is the regression equation used in this study. Equation No. 2 is the regression equation of Binnington, Sibbitt, and Geddes (1938).

It will be seen that the values calculated from the two regression equations are virtually identical and are in excellent agreement with the

observed values. This agreement is especially good considering the fact that the equation derived in this study is calculated from data obtained in eight different laboratories and with three different methods of estimating pigment content. The standard errors of prediction are likewise essentially the same, being 0.14 ppm. for this study and 0.17 ppm. for the data reported by Binnington, Sibbitt, and Geddes.

To estimate the precision of the two techniques, the duplicate results were employed to calculate the standard error of a single determination which was found to be 0.026 ppm. for either or both solvents. Thus both solvents yield equally precise results. The butanol procedure, however, gives a better differentiation between samples, since the spread between values is greater with the same experimental error.

When comparisons are drawn between the various methods of estimating pigment content in extracts, the results are not so favorable. Inspection of the mean values for all flours presented in Table I indicates that the photoelectric procedure yields the highest results, and the visual technique the lowest. While these differences are not very great, the inclusion of results known to be of doubtful accuracy would change the picture considerably, and even employing only results of presumed reliability, a difference still exists between methods which is statistically significant. In this connection, the results obtained by two collaborators employing KWSZ photoelectric photometers are of interest and are presented in Table III.

TABLE III

COMPARISON OF FLOUR CAROTENE VALUES, WATER SATURATED BUTANOL SOLVENT AND KWSZ PHOTOMETER

	Carotenoid pigment in flour, *ppm.*			
	A	B	Mean	General mean (Table I)
Flour				
A	0.36	0.47	0.42	0.82
B	0.64	1.96	0.85	1.56
C	0.41	0.58	0.50	1.02
D	1.11	2.18	1.65	2.92
E	1.27	2.69	1.98	3.40
F	1.27	2.65	1.96	3.45

It will be noted that the mean values are much lower than the general means in Table I, and also that poor agreement is shown between the two sets of results. Fundamentally there is no valid reason why such a discrepancy should exist, and it is believed that the explanation resides in the method of standardization employed. The significant differences between spectrophotometric, visual, and photoelectric results

are probably attributable to the same difficulty of standardization, and it would seem that this represents the major source of error in pigment determinations, since the precision of all three methods when calculated from the results of duplicate determinations is identical, the standard error of a single determination being again 0.026 ppm. in all cases.

Several of the collaborators commented favorably upon the rapid procedure, and six indicated that they are employing it as a routine test. Only one collaborator offered adverse criticism, and this referred only to the odor of the solvent, which he states necessitated working in a hood. Such practice does not appear to be general among those employing the method.

Summary

The Binnington-Geddes rapid method for determining flour pigments has been subjected to collaborative study in the instance of six flours and nine collaborators.

Tests were made in comparison with the standard A. A. C. C. procedure employing naphtha + alcohol solvent. Various methods of estimating the pigment content of the extracts obtained were employed, including spectrophotometric, visual, and photoelectric procedures.

The rapid method extracts more pigment, thus yielding results of a higher order of magnitude than the naphtha + alcohol procedure. Regression equations are presented enabling conversion of results by one solvent into the other and *vice versa*. The error of prediction of butanol values from naphtha + alcohol is ± 0.11 ppm. These equations enable comparison of prior results with those obtained by the rapid procedure.

It is shown that both methods yield relatively similar results regardless of the method employed for estimating pigment content or of the laboratory in which the tests are performed. Both techniques show high and equal precision, the standard error of a single determination being 0.026 ppm. for either or both procedures. The rapid method gives a somewhat better differentiation between samples.

A statistically significant difference was found between the various methods of estimating pigment content, which may be primarily due to variations in the mode of standardization. It is suggested that methods of standardization and estimation be investigated with a view to increasing the accuracy of this phase of flour pigment determinations.

Acknowledgments

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It is also desired to acknowledge the valuable assistance of Willis S. Hutchinson and R. I. Derby, who selected and prepared the samples and conducted the control storage tests, and of F. C. Hildebrand for assistance in statistical reduction of the data. This work was undertaken as a subproject of the Commitee on Methods of Analysis.

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COLLABORATIVE STUDY OF THE FERRICYANIDE METHOD FOR THE DETERMINATION OF DIASTATIC POWER OF MALT [1]

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(Read at the Annual Meeting, May 1940)

Last year's committee recommended that the activities for the present year be confined to a study of the determination of diastatic power. Previous work of the committee (Singruen, 1939) had showed that the ferricyanide method as modified by Anderson and Sallans (1937) gave less variation than the official method of the American Society of Brewing Chemists. Accordingly it was decided to carry out a comprehensive collaborative study of the ferricyanide method in the hope that statistical analysis of the data would give information on the variation in values obtained within any one laboratory and between laboratories.

Samples of three malts and a sample of soluble starch were sent to each of twelve collaborators with detailed directions for the procedure to be followed. Duplicate determinations were to be made on each malt on each of four days and all of the data reported, including standardizations of the solutions used.

[1] Report of the Malt Analysis Standardization Committee (1939–1940).
[2] Stationed at Madison, Wisconsin.

TABLE I

SUMMARY OF RESULTS ON COLLABORATIVE STUDY OF THE FERRICYANIDE METHOD FOR THE DETERMINATION OF DIASTATIC POWER OF MALTS—VALUES IN DEGREES LINTNER

	Collaborator											Mean collaborators	Range collaborators
	1	2	3	4	5	6	7	8	9	10	11		
Malt 1. Mean of 8 determinations	122.3	133.9	126.8	123.5	120.9	128.5	126.3	124.6	120.6	129.8	123.1	125.5	13.3
Range	3.8	3.4	3.2	4.8	4.5	4.3	3.0	1.9	1.6	2.3	3.1	3.3	—
Malt 2. Mean of 8 determinations	128.5	141.6	133.9	134.3	127.3	134.7	128.2	130.5	128.4	132.8	128.4	131.7	14.3
Range	5.1	4.3	4.7	13.7	10.4	5.4	6.9	2.0	3.9	1.8	4.3	5.7	—
Malt 3. Mean of 8 determinations	158.7	174.6	167.6	164.0	157.2	164.2	162.8	161.2	157.1	164.1	160.3	162.9	17.5
Range	8.3	5.4	5.0	14.4	18.7	5.4	9.0	5.1	4.6	2.8	6.5	7.7	—
Mean of malts	136.5	150.0	142.8	140.6	135.1	142.5	139.1	138.8	135.4	142.2	137.3	140.0	14.9
Mean range all malts	5.7	4.4	4.3	11.0	11.2	5.0	6.3	3.0	3.4	2.3	4.6	5.6	—
Range over days—mean all malts	3.1	2.1	1.0	9.0	6.1	4.4	3.3	0.8	1.4	0.8	1.7	3.1	—
Standard error of mean of duplicates, °L.	1.29	0.80	1.32	—	—	1.36	1.28	0.86	0.64	0.56	0.43	—	—

Results were submitted by eleven of the twelve collaborators, but the data from two laboratories reporting had to be omitted from the statistical analysis because some of the values requested were not obtained.

Presentation and Discussion of Data

A summary of the data, giving the means for collaborators and the range in the values making up the means, is given in Table I. An inspection of these data shows considerable variation in the values obtained by the different laboratories and in the range within the eight single determinations in the individual laboratories. When the values of Collaborator 2, which are consistently high, are not considered, the other ten laboratories can be divided roughly into a high and a low group. The agreement in diastatic power within the two separate groups is very good. The variation between duplicate determinations, as indicated by the standard error of means of duplicates, also divides the collaborators into two groups, but the placement of the laboratories in the groups is not the same by the two methods. In other words there appears to be no consistent association of low duplicate errors with either low or high diastatic-power values. A test of significance was applied to the means of the two groups distinguished by the duplicate errors and it was found that they did not differ significantly.

TABLE II

ANALYSES OF VARIANCE FOR COMBINED DATA

Variance due to	D.F.	Mean square
Samples	2	28,813.7**
Labs.	8	531.6**
Samples × Labs.	16	17.0

Note: In this and the following table, ** denotes that the 1% level, and * that the 5% level of significance is attained.

As all the laboratories did not place the malts in exactly the same relative positions it was necessary to resort to statistical analysis in order to determine the significance of the differences between laboratories. In this analysis, only the mean diastatic-power values over all days for each laboratory were used. The variations within laboratories were treated separately. The results of the pooled analysis of variance are shown in Table II. These data show that, in comparison with the mean square due to the changes in the relative positions of the samples from laboratory to laboratory the differences between samples and the differences

between laboratories attain a highly significant level. The value necessary to show significant differences between laboratories is 2.5° L.

The results of the analyses of variance within laboratories are shown in Table III. In five of the nine laboratories the variations between

TABLE III

ANALYSES OF VARIANCE FOR EACH LABORATORY

	Mean squares			
Laboratory	Samples 2 D.F.	Days 3 D.F.	S × D 6 D.F.	Duplicates 12 D.F.
9	2978.4**	2.3	2.4	0.9
1	3035.9**	12.1	3.6	3.3
11	3242.6**	3.8	8.9**	0.4
8	2965.7**	1.8	2.4	1.5
7	3375.2**	15.8	8.0	3.3
10	2893.8**	0.8	0.4	0.6
6	2921.0**	13.3*	0.9	3.6
3	3799.9**	1.5	1.9	3.5
2	3734.4**	6.0	3.4	1.3
Mean	3216.7	6.4	3.5	2.0

days are significantly greater than the variation between duplicates, but in only one laboratory (No. 6) is the effect of days significantly greater than the effect of interaction between samples and days. In one laboratory (No. 11) the variance due to interaction between malts and days is significantly greater than that due to differences between duplicates. Under ideal conditions the mean squares due to days, interaction between days and malts and duplicates should be of the same order and should be as small as possible. It therefore appears necessary for each laboratory to investigate its own sources of error.

The differences between laboratories in the diastatic-power values do not appear to be associated with any particular source of error within laboratories and the greatest error apparent in this test is the interaction between malts and laboratories. However, it is not unlikely that this error would be reduced in future tests conducted after the laboratories had investigated their own errors.

In two of the collaborating laboratories, different samples of Merck's Reagent Soluble Starch were used in addition to the sample sent out with the malts. In one laboratory significantly different values were obtained with the two starches, while in the second the differences between starch samples was not significant. This indicates that where two soluble starches differ sufficiently in their characteristics, their use will introduce an appreciable error in the results obtained. In collabo-

rative work on diastatic-power determinations a uniform sample of starch should be supplied to all collaborators.

The normality of the thiosulphate solutions was requested in this study and it afforded evidence of the magnitude of the error which might be ascribed to this one factor if the solutions were not standardized and the correction applied. The maximum variation between the reported values from ten laboratories was 0.0017, which if not corrected would be equivalent to approximately 4.0° Lintner based on the means of the three malts.

The unexplained variations in the values obtained in this collaborative study are still greater than desirable, but they are less than those obtained with any other method that has been studied collaboratively by this committee. Therefore the committee proposes the following recommendations:

That the ferricyanide method for the determination of diastatic power be adopted as tentative as well as the method of the American Society of Brewing Chemists (Coleman, 1936).

That certain phases of the method be studied further in an effort to reduce the variation and to standardize the procedure to a greater degree.

That the major emphasis of the committee be directed toward a study of the available methods for the determination of alpha-amylase, with the purpose of finding or developing an acceptable method.

Acknowledgments

The chairman wishes to express his appreciation to the members of the committee and to the collaborators for their excellent cooperation in this study and to acknowledge the assistance of W. O. S. Meredith in the statistical analysis and preparation of the report.

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A COMPARISON BETWEEN THE OFFICIAL AIR-OVEN MOISTURE METHOD AND THE RAPID ALUMINUM-PLATE METHOD [1]

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(Read at the Annual Meeting, May 1940)

The aluminum-plate method used in the tests to be reported was that described by Sandstedt.[2] The official method is that which is given in the A. A. C. C. book of methods. The official method was interpreted to mean one hour of total time for the sample in the oven. This interpretation was used for two reasons: first, it is the interpretation which was used in the development of the rapid method and second, it is the interpretation placed upon the method by a majority of control chemists using the procedure.

The problem of comparing the two methods was approached with the idea of determining the effects of position of the sample in the oven and spreads within the methods as well as between the methods.

Every possible precaution was taken to eliminate errors in sampling and the sequence of weighing of each sample was recorded. Three flour samples were used and the results obtained are summarized in Table I.

TABLE I

COMPARISON OF OFFICIAL AND ALUMINUM PLATE METHODS WITH FLOUR SAMPLES

Sample number	Official method			Aluminum plate method		
	Number of determinations	Mean	Range	Number of determinations	Mean	Range
		%	%		%	%
1	10	14.36	0.40	10	14.42	0.20
2	10	10.69	0.25	10	10.69	0.10
3	32	10.63	0.30	32	10.62	0.20

Two samples of ground wheat were used for a comparison of the methods on this material. Precautions were again taken to insure freedom from sampling differences. The time for the aluminum-plate method was varied from 15 to 40 minutes. The results obtained are summarized in Table II.

The writer believes that the average operator fails to recognize the significance and magnitude of errors that are obtained as a result of

[1] Journal Series No. 269, Nebraska Agricultural Experiment Station.
[2] R. M. Sandstedt, The rapid determination of moisture, Cereal Chem. 15: 813–815, 1938.

TABLE II

COMPARISON OF OFFICIAL AND ALUMINUM PLATE METHODS WITH GROUND WHEAT SAMPLES

Sample number	Number of determinations	Mean	Range	Time	Number of determinations	Mean	Range
		%	%	*Min.*		%	%
1	20	17.57	0.50	15	10	17.18	0.40
				20	20	17.40	0.35
				25	10	17.38	0.20
				40	10	17.68	0.30
2	15	16.02	0.25	20	15	15.84	0.35
				40	15	16.06	0.25

loss of moisture while weighing a series of samples from the same container. Errors greater than those inherent in the method may be obtained because of losses of moisture during low humidity periods. This is perhaps particularly true of the Southwest where the relative humidity in the laboratory is abnormally low during the winter months. However, even in other parts of the country where low temperatures prevail, air contains very little moisture after being raised from outdoor temperatures to normal room temperatures. With this thought in mind the average results obtained from samples weighed in sequence of one to sixteen were plotted against the position in the sequence. The results are represented graphically in Figure 1.

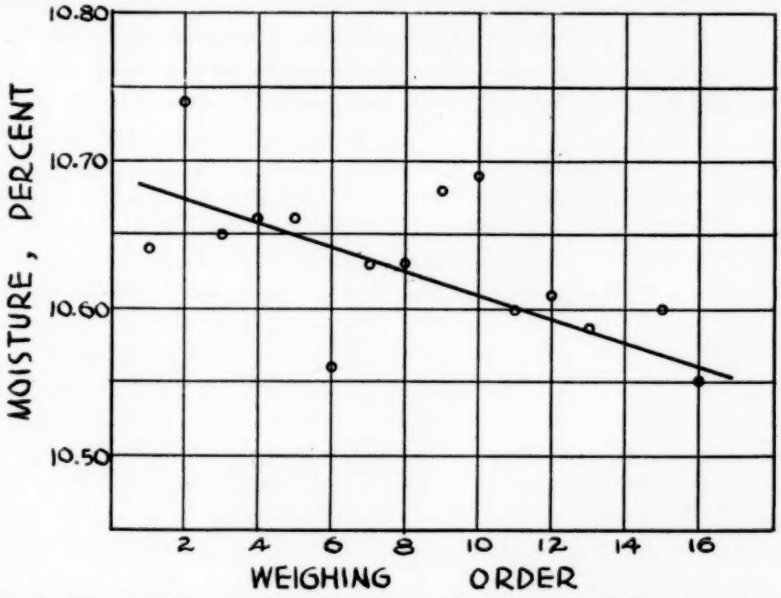

Fig. 1. The relation between moisture loss and the number of samples weighed from a container.

Conclusions

The two methods when used on flour samples gave results which are in agreement well within the limits of error of either method.

There is a lesser tendency for variation caused by position in the oven when the rapid aluminum plate method is used than where the air oven is used. Care must be taken not to place samples too near the front of the oven when the official air method is used.

The rapid method does not give satisfactory results on samples of ground wheat. Preliminary indications are that ground wheat samples must be heated for a period of time closer to 40 minutes than the formerly recommended 20 minutes if comparable results are to be obtained.

Recommendations

It is recommended that the rapid method be considered comparable to the official method (if the above interpretation of the air-oven method is held to be correct) and that further work be done on the determination of moisture on ground wheat samples. It is entirely possible that the use of a smaller sample would allow the determination to be made with comparable results in as short a period of time as that used for flour samples.

DETERMINATION OF MOISTURE IN FLOUR AND GROUND WHEAT—A COLLABORATIVE COMPARISON OF THE OFFICIAL AIR-OVEN AND RAPID ALUMINUM-PLATE METHODS[1]

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(Read at the Annual Meeting, May 1940)

In accordance with the recommendation of the 1938–39 Committee on Methods of Analysis, the comparison between the rapid aluminum-plate method for determining moisture in flour and wheat (Sandstedt, 1938) and the "official" air-oven method (drying for one hour at 130°C., was continued. Previous work suggested that agreement within practical limits was possible when flour was dried for 15 minutes on the aluminum plate. Results with ground wheat dried for 20 minutes did not agree with those obtained in the air oven.

Twenty-six collaborators were enlisted whose laboratories were equipped with standard makes of air ovens and the necessary cast or cold rolled aluminum plates one-half inch or more in thickness. To reduce variables, specific directions were given for standardizing equipment and handling samples. Four 2.000-gram subsamples were weighed from each. The first and third subsamples were dried by the air-oven method, the second and fourth by the rapid aluminum-plate method. Additional 2.000-gram samples were supplied as needed to make each oven load consist of eight samples, on the assumption that the objective of speed is defeated when a larger number of samples are accumulated before drying is started.

Collaborators observed the following general precautions: to have open all oven vents, to avoid large temperature drops by loading the oven quickly, to avoid placing samples under the pilot light, to place covers atilt on their respective dishes during drying, to replace covers promptly while dishes were still in the oven, to cool dishes in a desiccator, and to reweigh them soon after they reached room temperature (allowing not more than 20 minutes for cooling after taking dishes from the drying oven).

For making the "official" air-oven tests, the ovens were regulated to give a temperature of 130°C. (plus or minus 3°) measured by a thermometer having the tip of the bulb level with the top of the moisture dishes but not directly over a sample. Neither an aluminum plate nor a forced draft was used. Collaborators dried the dishes, covers, and con-

[1] Subcommittee report of the 1939–40 Committee on Methods of Analysis.

tents for one hour, beginning when the temperature of the oven regained 127°C.

The instructions applying specifically to the aluminum-plate method were: to place the half-inch (or more) thick aluminum plate on the lowest shelf support, to regulate the oven so that the temperature of the aluminum plate was 140°C. (plus or minus 1°) measured by a thermometer (the bulb of which rested on the bottom of a small can containing fine sand), and to dry the dishes, covers, and contents for a total of 15 minutes for flour and 20 minutes for ground wheat.

The first, middle, and last individual samples drawn from each general sample were retained and analyzed in duplicate in our laboratory on the day specified to the collaborators for their tests. These six results on each general sample were in excellent agreement, and reflected the great care used in preparing the samples.

Table I shows the moisture range of the seven flours used and the apparent close agreement between the averages obtained by the two methods.

TABLE I

AVERAGES OF MOISTURE DETERMINATIONS BY EACH METHOD ON FLOUR

Sample No.	(1) AOAC av.	(2) A. P. av.	Deviation of (2) from (1)
5	9.71	9.66	−.05
6	12.61	12.53	−.08
7	12.13	12.08	−.05
8	12.10	12.04	−.06
9	13.06	12.99	−.07
10	8.95	8.92	−.03
11	11.74	11.70	−.04

Samples 5 to 8 were soft-wheat flours; 9 to 11, hard-wheat flours. It may be observed that while the differences between the averages are small (being from 0.03% to 0.08% moisture), all by the aluminum-plate method are lower than by the air-oven method.

In Table II similar data are given for six samples of ground wheat. Differences between corresponding averages, all negative, are larger than those for flour in spite of the 20 minutes' drying on the aluminum plate.

The individual results obtained by all collaborators on all samples are summarized in Table III, which shows, in increments of one-tenth percent, the deviation of results by the rapid aluminum-plate method from the corresponding 130°C. air-oven results. The averages of duplicates in 96 comparative tests on soft-wheat flours showed an average

TABLE II

AVERAGES OF MOISTURE DETERMINATIONS BY EACH METHOD ON GROUND WHEAT

Sample No.	(1) AOAC av.	(2) A. P. av.	Deviation of (2) from (1)
12	8.66	8.57	−.09
13	8.82	8.71	−.11
14	6.38	6.19	−.19
15	8.64	8.50	−.14
16	13.71	13.58	−.13
17	6.30	6.12	−.18

TABLE III

SHOWING PERCENT OF TESTS DEVIATING FROM 130°C. AIR-OVEN RESULTS

Increments 0.1% each	Soft-wheat flour	Hard-wheat flour	Ground wheat
0.0 to 0.1	54.2	56.8	29.8
0.1 to 0.2	31.3	18.9	27.5
0.2 to 0.3	10.4	13.5	20.7
0.3 to 0.4	3.1	6.8	13.0
Over 0.4	1.0	4.0	9.0
Av. dev.	0.116	0.134	0.204
No. tests	96	74	131

deviation of less than 0.12% of moisture. Of these 54.2% deviated by 0.10% or less and a total of 85.5% by 0.20% or less from the 130°C. air-oven method. The averages of duplicates in 74 comparative tests on hard-wheat flours showed an average deviation of less than 0.14% moisture. Of these 58.6% deviated by 0.10% or less, and a total of 75.5% by 0.20% or less, from the 130°C. air-oven method.

The data shown in Table III are displayed in a different form in Table IV to emphasize that although a majority of the aluminum-plate-

TABLE IV

SHOWING PERCENT OF TESTS DEVIATING FROM 130°C. AIR-OVEN RESULTS

Increments 0.1% each	Soft-wheat flour	Hard-wheat flour	Ground wheat
−0.4 or less	3.1	8.1	17.5
−0.3	10.4	10.8	19.1
−0.2	21.9	12.2	20.6
−0.1	20.8	27.0	16.8
0.0	6.3	5.4	2.3
0.1	27.1	24.3	10.7
0.2	9.4	6.8	6.9
0.3 or more	1.0	5.4	6.1

method results on flour agree with the air-oven method within 0.20%, there were more negative than positive deviations, as was indicated by the small average negative differences reported in Table I. Specifically, Table IV shows that 6.3% of the corresponding determinations on soft-wheat flour were in perfect agreement, whereas 42.7% had a negative deviation of less than 0.20% moisture contrasted with the 36.5% which had a positive deviation of up to 0.20% moisture, showing a total of 85.5% within plus or minus 0.20%. Also, it shows for the hard-wheat flours that 5.4% of the corresponding determinations were in perfect agreement, whereas 39.2% had a negative deviation of less than 0.20% moisture, while 31.1% had a positive deviation up to 0.20% moisture, giving a total of 75.7% within plus or minus 0.20%.

The principal disadvantage of the aluminum-plate method as now specified appears to be the difficulty of accurately measuring the temperature of the aluminum plate itself. Several collaborators have proposed replacing the can of sand surrounding the thermometer bulb by either a mercury well or a can containing fine aluminum shot. This should be tried as it probably would improve agreement between collaborators.

As reported by Sandstedt (1938) one of the chief advantages of using the aluminum plate is the even distribution of heat over the entire shelf area which in effect increases the useful capacity of any drying oven (air or vacuum) because even the spaces near the door and under the pilot lamp can be used. When the aluminum plate is used small differences from the specified temperature do not affect seriously the accuracy of results because rate of heat transfer to the samples remains high.

The fact that 85.5% of the soft-wheat-flour results and 75.7% of the hard-wheat agree within 0.20% (as between the two methods) indicates that the aluminum-plate method can be made to function well enough to be used for mill control testing. In any one laboratory, these variations can be reduced by careful calibration with respect to the official method. The experimental error between collaborators using either method in this study was found to be not significantly greater than that reported by Davis and Wise (1933) in their analysis of the systematic error experienced in collaborative work by members of the Pioneer Section.

Therefore we recommend the rapid aluminum-plate method, 140°C. for 15 minutes, as an alternative method for the rapid determination of moisture in flour, and on this basis we suggest that no further collaborative comparisons be made on flour. The results with ground wheat are not satisfactory. As shown in Table III, only 29.8% of the results

agree within 0.10% and only 57.3% within 0.20%. The average deviation of the 131 comparisons is slightly more than 0.20% moisture. In Table IV, 74.0% of the results on ground wheat are seen to have a negative deviation. However, application of the rapid method to ground wheat cannot yet be condemned because it probably can be made to give results equivalent to those obtained by the official 130°C. one-hour method. Some may question the use of the 130°C. one-hour drying method for moisture in ground wheat. While no specific references appear in either the A. O. A. C. or the A. A. C. C. books of methods, the U. S. Department of Agriculture, Bureau of Agricultural Economics, issued a Service and Regulatory Announcement No. 147, April, 1935, describing an approved procedure for making these tests on ground wheat, and the 1934–35 A. A. C. C. Committee on Methods of Analysis recommended "that the vacuum-oven and air-oven (130°C. for 1 hour) methods be continued as the official methods for wheat feeds as well as flour."

Assuming the air-oven method as a reference point in testing wheat, we see no difficulty in further modifying the proposed 140°C. aluminum-plate method by increasing the drying time until equivalent results are obtained. Therefore we recommend that further work be done on ground wheat in a designated laboratory to determine new specifications to be subjected later to collaborative study.

It should always be borne in mind that the literature contains many references to probable sources of error in moisture testing. Bailey (1914) discussed the "non-uniformity of drying oven temperatures." Lanning (1935) showed that a poorly designed thermostat contributed to uneven temperatures in a small drying oven. He recommended defining the position of the thermometer bulb in relation to the samples and the time to be allowed for the oven to regain 130°C. Treloar and Sullivan (1935) in studying the variability of data obtained on feeds by the vacuum oven, air oven at 130°C. for one hour, and air oven at 135°C. for two hours, concluded that "there is no demonstrable gain in consistency through using the higher temperature and longer time in the air oven." Davis (1935) found that different oven ventilation conditions had little effect on the results by the air-oven method, 130°C. for one hour.

In view of these many papers and the commonplaceness of the moisture test, it may appear superfluous repetition to say that the fundamentals of what is essentially an empirical, physical test frequently are ignored. Therefore, by way of emphasis, we recommend to the Committee on Revision of Cereal Laboratory Methods:

1. Clarification of the language of the method to show that: "The one-hour drying period begins when the temperature of the oven regains 130°C." (This statement, according to Dr. Henry A. Lepper, editor, will appear in the forthcoming edition of *Methods of Analysis, A. O. A. C.*)

2. A statement specifying where the tip of the thermometer bulb shall be placed in relation to the samples being dried.

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NOTE ON MODIFICATION OF THE A. A. C. C. METHODS FOR THE DETERMINATION OF TOTAL AND RESIDUAL CO_2 IN BAKING POWDERS AND TOTAL CO_2 IN SELF-RISING FLOURS

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This note is presented with the thought that the modifications as used in our laboratories in the routine testing of CO_2 in baking powders and self-rising flours may help other investigators to secure reproducible results and may also save considerable time.

Determination of the residual CO_2 in baking powders [1] involves a step wherein a rather thick, gelatinous suspension is boiled for one minute. We have found that often there is insufficient water present to permit the starch to become dextrinized and caramelized, in which case high CO_2 values are obtained. A simple preventive measure is the addition of 2 to 3 cc. of water just before boiling. A heating period slightly longer than one minute is not necessary. As much as 5 cc. of added water has been found to have no adverse effect on the recovery of CO_2.

A modification has been used also in the gasometric measurement of residual and total CO_2 in baking powders and of CO_2 in self-rising flours. Methods for total CO_2 include a step in which from 5 to 10 minutes of standing is required after the CO_2 has been liberated and before the final reading of the gas burette. This is to insure equalization of the temperature of the gases in the flask and the burette. A simple way to reduce the time for each determination is to use acid which has been adjusted in temperature to about 1°C. below room temperature when testing for residual or total CO_2 in baking powders and about 2°C. when testing self-rising flours for total CO_2. A thermometer inserted through a third hole in the stopper holding the CO_2 flask will indicate the temperature of the suspension. Acid sufficiently below room temperature is used so that after shaking for the prescribed time, the temperature within the flask will have attained that of the gas burette, *i.e.*, room temperature. Gas volume may then be recorded immediately with little loss of time. It has been observed that upon different days slightly different temperature adjustments of the acid are required.

[1] Cereal Laboratory Methods, Chapter 9, Methods 2 and 3, pp. 114–117, 1935.